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(54) Title: NOVEL EXPRESSION VECTORS AND USES THEREOF

(57) Abstract: The present invention relates to novel vectors, to DNA vaccines and gene therapeutics containing said vectors, to methods for the preparation of the vectors and DNA vaccines and gene therapeutics containing the vectors, and to therapeutic uses of said vectors. More specifically, the present invention relates to novel vectors comprising (a) an expression cassette of a gene of a nuclear-anchoring protein, which contains (i) a DNA binding domain capable of binding to a specific DNA sequence and (ii) a functional domain capable of binding to a nuclear component and (b) a multimerized DNA sequence forming a binding site for the anchoring protein, and optionally (c) one or more expression cassettes of a DNA sequence of interest. In particular the invention relates to vectors that lack a papilloma virus origin of replication. The nuclear-anchoring protein might be the E2 protein of Bovine Papilloma Virus type 1 or Epstein-Barr Virus Nuclear Antigen 1. The invention also relates to vectors that lack an origin of replication functional in a mammalian cell. The invention further relates to methods for expressing a DNA sequence of interest in a subject.

NOVEL EXPRESSION VECTORS AND USES THEREOF

1. FIELD OF THE INVENTION

The present invention relates to novel vectors, to DNA vaccines and gene therapeutics containing said vectors, to methods for the preparation of the vectors and DNA vaccines and gene therapeutics containing the vectors, and to therapeutic uses of said vectors. More specifically, the present invention relates to novel vectors comprising (a) an expression cassette of a gene of a nuclear-anchoring protein, which contains (i) a DNA binding domain capable of binding to a specific DNA sequence and (ii) a functional domain capable of binding to a nuclear component and (b) a multimerized DNA forming a binding site for the anchoring protein of a nuclear-anchoring protein, and optionally (c) one or more expression cassettes of a DNA sequence of interest. In particular the invention relates to vectors that lack a papilloma virus origin of replication. The invention also relates to vectors that lack an origin of replication functional in a mammalian cell. The invention further relates to methods for expressing a DNA sequence of interest in a subject.

2. BACKGROUND OF THE INVENTION

Transfer of autologous or heterologous genes into animal or human organisms with suitable vectors is emerging as a technique with immense potential to cure diseases with a genetic background or to prevent or cure infectious diseases. Several types of viral and non-viral vectors have been developed and tested in animals and in human subjects to deliver a gene/genes that are defective by mutations and therefore non-functional. Examples of such vectors include Adenovirus vectors, Herpes virus vectors, Retrovirus vectors, Lentivirus vectors and Adeno-associated vectors.

Vaccination has proven to be a highly effective and economical method to prevent a disease caused by infectious agents. Since the introduction of the Vaccinia virus as an attenuated vaccine against the smallpox virus (Variola), vaccines against a multitude of human pathogens have been developed and taken into routine use. Today small pox has been eradicated by vaccinations and the same

is to be expected shortly for the poliovirus. Several childhood diseases, such as pertussis, diphtheria and tetanus, can be effectively prevented by vaccinations.

In general, the most successful viral vaccines are live avirulent mutants of the disease-causing viruses. The key to the success of this approach is the fact
5 that a living virus targets the same organs, the same type and similar number of cells, and therefore, by multiplying in the recipient, elicits a long-lasting immune response without causing the disease or causing only a mild disease. In effect, a live attenuated vaccine produces a subclinical infection, the nature's own way of immunizing. As a result, a full immune response will be induced, including hu-
10 moral, cellular and innate responses, providing a long lasting and sometimes a life-long immune protection against the pathogen.

Although live attenuated vaccines are most potent, they can cause harmful side effects. Thus, an attenuated viral vaccine can revert to a virulent strain or in cases where the attenuated virus is apathogenic in adults it can still cause a dis-
15 ease in infants or in disabled persons. This is true in the case of viruses causing chronic infections, such as Human Immunodeficiency Virus type 1 and 2. Vac-
cines composed of viral and bacterial proteins or immunogenic peptides are less likely to cause unwanted side effects but may not be as potent as the live vac-
cines. This is especially the case with vaccines against microbes causing chronic
20 infections, such as certain viruses and intracellular bacteria.

The strength and type of immune response is, however, also dependent on how the viral proteins are processed and how they are presented to the im-
mune system by antigen presenting cells (APCs), such as macrophages and dendritic cells. Protein and peptide antigens are taken up by APCs via endocyto-
25 sis, processed to small immunogenic peptides through an endosomal pathway and presented to T-lymphocytes (T-cells) by MHC (major histocompatibility com-
plex) class II antigens [in man HLAs (human leukocyte antigens) class II]. In con-
trast, proteins synthesized de novo in APCs or in possible target cells for an im-
mune response, will be processed through a cytoplasmic pathway and presented
30 to T-cells by MHC class I antigens (in man HLAs class I). In general, the presen-
tation of immunogenic peptides through the class II pathway will lead to the acti-
vation of the helper/inducer T-cells, which in turn will lead to the activation of B-

cells and to antibody response. In contrast, presentation through class I MHC favors the induction of cytotoxic T-lymphocytes (CTLs), which are capable of recognition and destruction of virally infected cells.

In early 1990's, a method to mimic the antigen processing and presentation that was normally achieved by live attenuated vaccines was introduced [Ulmer, J.B. et al Science 259 (1993) 1745-1749]. It was shown that an injection of eukaryotic expression vectors in the form of circular DNA into the muscle induced take-up of this DNA by the muscle cells (and probably others) and was able to induce the expression of the gene of interest, and to raise an immune response, especially a cellular immune response in the form of CTLs, to the protein encoded by the inserted gene. Since that observation, DNA immunization has become a standard method to induce immune responses to foreign proteins in experimental animals and human studies with several DNA vaccines are underway.

Generally, the DNA vectors used in these vaccine studies contain a cloning site for the gene of interest, a strong viral promoter, such as the immediate early promoter of the CMV virus, in order to drive the expression of the gene of interest, a polyadenylation region, and an antibiotic resistance gene and a bacterial replication origin for the propagation of the DNA vector (plasmid) in bacterial cells.

With the vectors described above it is possible to obtain a detectable level of expression of the gene of interest after administering the vector to experimental animals or to humans, either by a direct injection to muscle or to skin with a particle bombardment technique or by applying the vector in a solution directly to mucous membranes. However, the expression obtained by these vectors is short lived: the vectors tend to disappear from the transfected cells little by little and are not transferred to daughter cells in a dividing cell population. The short-term expression of the gene of interest and limited number of cells targeted are probably the major reasons, why only temporary immune responses are observed in subjects immunized with DNA vectors described above. Thus, for example, Boyer et al. observed only temporary immune responses to HIV-1 Env and Rev proteins in

human subjects, who were immunized several times with a vector similar to the those described above [Boyer, J. D., J Infect Dis 181 (2000) 476 – 483].

There is a growing interest in developing novel products useful in gene therapy and DNA vaccination. For instance papilloma virus vectors carrying the 5 expression cassette for the gene of interest have been suggested to be useful candidates.

To date more than 70 subtypes of human papilloma viruses (HPVs) and many different animal papilloma viruses have been identified [zur Hausen, H. and de Villiers E., Annu Rev Microbiol 48 (1994) 427 – 447; Bernard, H., et al., Curr 10 Top Microbiol Immunol 186 (1994) 33 – 54]. All papilloma viruses share a similar genome organization and the positioning of all of the translational open reading frames (ORFs) is highly conserved.

Papilloma viruses infect squamous epithelial cells of skin or mucosa at different body sites and induce the formation of benign tumors, which in some cases 15 can progress to malignancy. The papilloma virus genomes are replicated and maintained in the infected cells as multicopy nuclear plasmids. The replication, episomal maintenance, expression of the late genes and virus assembly are tightly coupled to the differentiation of the epithelial tissue: the papilloma virus DNA episomal replication takes place during the initial amplificational replication and 20 the second, i.e. latent, and the third, i.e. vegetative, replications in the differentiating epithelium [Howley, P. M.; Papillomavirinae: the viruses and their replication. In Virology, Fields, B. C., Knipe, D. M., Howley, P. M., Eds., Lippincott-Raven Publishers, Philadelphia, USA, 1996, 2. Edition, p. 2045 – 2076].

Two viral factors encoded by the E1 and E2 open reading frames have 25 been shown to be necessary and sufficient for the initiation of the DNA replication from the papilloma virus origin in the cells [Ustav, M. and Stenlund, A., EMBO J 10 (1991) 449 - 57; Ustav, M., et al., EMBO J 10 (1991) 4321 - 4329; Ustav, E., et al., Proc Natl Acad Sci USA 90 (1993) 898 - 902].

Functional origins for the initiation of the DNA replication have been defined for BPV1 [Ustav, M., et al., EMBO J 10 (1991) 4321 – 4329], HPV1a 30 [Gopalakrishnan, V. and Khan, S., supra], HPV11 [Russell, J., Botchan, M., J Virol 69 (1995) 651 –660], HPV18 [Sverdrup, F. and Khan, S., J Virol 69 (1995)

- 1319 - 1323:Sverdrup, F. and Khan, S., J Virol 68 (1994) 505-509] and many others. Characteristically, all these origin fragments have a high A/T content, and they contain several overlapping individual E1 protein recognition sequences, which together constitute the E1 binding site [Ustav, M., et al., EMBO J 10 (1991) 5 4321 – 4329; Holt, S., et al., J Virol 68 (1994) 1094 - 1102; Holt, S. and Wilson, V., J Virol 69 (1995) 6525 - 3652; Sedman, T., et al. J Virol 71 (1997) 2887 - 2996]. In addition, these functional origin fragments contain an E2 binding site, which is essential for the initiation of DNA replication *in vivo* in most cases (Ustav, E., et al., *supra*). The E2 protein facilitates the first step of the origin recognition 10 by E1. After the initial binding of monomeric E1 to the origin the multimerization of E1 is initiated. This leads to the formation of the complex with the ori melting activity. It has been suggested that E2 has no influence on the following stages of the initiation of the DNA replication [Lusky, M., et al., Proc Natl Acad Sci USA 91 (1994) 8895-8899].
- 15 The BPV1 E2 ORF encodes three proteins that originate from selective promoter usage and alternative mRNA splicing [Lambert, P., et al., Annu Rev Genet 22 (1988) 235 – 258]. All these proteins can form homo- and heterodimers with each other and bind specifically to a 12 bp interrupted palindromic sequence 5'-ACCNNNNNNGGT-3' [Androphy, E., et al., Nature 325 (1987) 70 – 739].
- 20 There are 17 E2 binding sites in the BPV1 genome and up to four sites in the HPV genomes, which play a crucial role in the initiation of viral DNA replication (Ustav, E., et al., *supra*) and in the regulation of viral gene expression (Howley, P. M., Papillomavirinae: the viruses and their replication, in *Virology*, Fields, B. C., Knipe, D. M., Howley, P. M., Eds., Philadelphia: Lippincott-Raven Publishers, 1996. 2. edition, p. 2045 – 2076). Structural and mutational analyses have 25 revealed three distinct functional domains in the full size E2 protein. The N-terminal part (residues 1 to 210) is an activation domain for transcription and replication. It is followed by the unstructured hinge region (residues 211 to 324) and the carboxy-terminal DNA binding-dimerization domain (residues 325 to 410)
- 30 [Dostatni, N., et al., EMBO J 7 (1988) 3807 – 3816; Haugen, T., et al. EMBO J 7 (1988) 4245 – 4253; McBride, A., et al., EMBO J 7 (1988) 533 – 539; McBride, A., et al., Proc Natl Acad Sci USA 86 (1989) 510-514]. On the basis of X-ray crys-

tallographical data, the DNA binding-dimerization domain of E2 has a structure of a dyad-symmetric eight-stranded antiparallel beta barrel, made up of two identical "half-barrel" subunits [Hegde, R., et al., Nature 359 (1992) 505 – 512; Hegde, R., J Nucl Med 36(6 Suppl) (1995) 25S - 27S]. The functional elements of the trans-
activation domain of E2 have a very high structural integrity as confirmed by mu-
tational analysis [Abroi, A., et al., J Virol 70 (1996) 6169 – 6179; Brokaw, J., et
al., J Virol 71 (1996) 23 – 29; Grossel, M., et al., J Virol 70 (1996) 7264 – 7269;
Ferguson, M. and Botchan, M., J Virol 70 (1996) 4193-4199] and by X-ray crystal-
lography [Harris, S., and Botchan, M.R., Science 284 (1999) 1673-1677 and Ant-
son, A. et al., Nature 403 (2000) 805-809]. In addition, X-ray crystallography
shows that the N-terminal domain of the E2 protein forms a dimeric structure,
where Arg 37 has an important function in dimer formation (Antson, A., et al., su-
pra).

As has been described previously, bovine papillomavirus type 1 E2 protein
in trans and its multiple binding sites in cis are both necessary and sufficient for
the chromatin attachment of the episomal genetic elements. The phenomenon is
suggested to provide a mechanism for partitioning viral genome during viral infec-
tion in the dividing cells [Ilves, I., et al., J Virol. 73 (1999) 4404-4412].

None of the papilloma vectors or other vectors disclosed so far fulfills the
criteria and requirements set forth for an optimal vaccine, which are the same for
DNA vaccines and for conventional vaccines. (It should be noted that these re-
quirements are preferred but not necessary for use as a vaccine.) First, an opti-
mal vaccine must produce protective immunity with minimal adverse effects. Thus
the vaccine should be devoid of components, which are toxic and/or cause symp-
toms of the disease to the recipient. Second, an optimal vaccine must induce a
pathogen-specific immune response, i.e. it must elicit a strong and measurable
immune response to the desired pathogen without causing an immune response
to other components of the vaccine. These two requirements imply that a vector
to be used as a DNA vaccine should optimally only express the desired gene(s)
and optimally should not replicate in the host or contain any sequences homolo-
gous with those of the recipient, since nucleotide sequences that are homologous
between the vector and the host's genome may effect the integration of the vector

into the host's genome. Third, an optimal vaccine must induce a right type of immune response; i.e. it must raise both humoral and cellular immune responses in order to act on the intracellular and extracellular pathogen. Finally, an optimal vaccine must be stable, i.e. it must retain its potency for a sufficiently long time in
5 the body to raise the immune response in a vaccine formulation for use in various demanding circumstances during storage and preparation. Additionally, vaccines should be of reasonable price. Further, the route and the method of inoculation are important considerations for optimizing a DNA immunization.

When developing a DNA vaccine the stability of the expression of the de-
10 sired gene is sometimes a major problem. Thus, the maintenance function or the persistance of the vector in the recipient cell has been focused on in the prior art, however, often at the cost of the safety. For example, Ohe, Y., et al.] [Hum Gene Ther 6(3) (1995) 325-333] disclose a papilloma virus vector capable of stable, high-level gene expression, which is suggested for use in gene therapy. Trans-
15 forming early genes E5, E6, and E7 have been deleted from said vector, but it still contains nucleotide sequences encoding other papilloma viral genes, such as the E1 and E2 genes, which are involved in the replication of the virus. Thus, the vector produces several other papilloma proteins, which may elicit undesired immune responses and which induce a risk of the vector's integration in the recipient. Al-
20 so, the vector is replicable, since it contains the E1 gene. Additionally, it is large in size and therefore subject to bacterial modification during preparation.

International Patent Application PCT/EE96/00004 (WO 97/24451) dis-
25 closes vectors capable of a long-term maintenance in a host cell and methods.us-
ing such vectors for obtaining long-term production of a gene product of interest
in a mammalian host cell, which expresses E1 and E2. These vectors contain a
minimal origin of replication of a papilloma virus (MO), a Minichromosome Main-
tenance Element (MME) of a papilloma virus and a gene encoding said gene
product, the MO and MME consisting of a DNA sequence different from the natu-
ral papilloma virus sequence, and in some embodiments the E1 gene. Addi-
30 tionally, vectors containing an MME consisting essentially of ten E2 binding sites are
disclosed in some examples. These vectors require the presence of the E1 pro-
tein either in the host or in the vector for the expression. This imparts the replica-

tion function to the vectors. These vectors also express the E1 protein in addition to the gene of interest and the E2 protein and contain sequences, such as rabbit β-globin sequences, which are partially homologous to human sequences causing a serious risk of integration to human genome, which reduces the potential of 5 these vectors as DNA vaccines. Additionally, the vectors are unstable due to their size (ca 15 kb): at the preparation stage in a bacterial cell, the bacterial replication machinery tends to modify the vector by random slicing of the vector, which leads to unsatisfactory expression products including products totally lacking the gene of interest.

10 International Patent Application PCT/EE96/00004 (WO 97/24451) further discloses that E1 and E2 are the only viral proteins necessary for the episomal long-term replication of the vectors. Additionally, the maintenance function of the BPV1 genome is associated with the presence of minimal ori (MO), which is stated to be necessary, although not sufficient, for the long-term persistence or 15 the stable maintenance of the vectors in the cells. In addition, the cis-elements, i.e. the Minichromosome Maintenance Elements of the BPV1, are stated to be required for the stable replication of BPV1. In particular, multimeric E2 binding sites (E2BS) are stated to be necessary for the stable maintenance of the vectors.

There is a clear need for improved novel vectors, which would be useful as
20 DNA vaccines.

An object of the invention is therefore to provide novel vectors, which are capable of a long-term maintenance in a large and increasing number of different cells of the host's body and thereby capable of providing a stable expression of the desired antigen(s).

25 Another object of the invention is to provide novel vectors, which are maintained for a long period of time in the cells that originally received the vector and transferred it to the daughter cells after mitotic cell division.

Yet another object of the invention is to provide novel vectors, which express in addition to the gene or genes of interest preferably only a gene necessary for a long-term maintenance in the recipient cells and thus are devoid of 30 components that are toxic or cause symptoms of the disease to the recipient.

A further object of the invention is to provide novel vectors, which mimic attenuated live viral vaccines, especially in their function of multiplying in the body, without inducing any considerable signs of disease and without expressing undesired proteins, which may induce adverse reactions in a host injected with the
5 DNA vaccine.

Still a further object of the invention is to provide novel vectors, which do not replicate in the recipient.

Still another object of the invention is to provide novel vectors, which induce both humoral and cellular immune responses when used as DNA vaccines.

10 Yet another object of the invention is to provide novel vectors, which are suitable for a large-scale production in bacterial cell.

Yet another object of the invention is to provide novel vectors, which are not host specific and thus enable the production in various bacterial cells.

An additional object of the invention is to provide novel vectors, which are
15 useful as carrier vectors for a gene or genes of interest,

A further object of the invention is to provide novel vectors, which are useful in gene therapy and as gene therapeutic agents and for the production of macromolecular drugs in vivo.

20 3. SUMMARY OF THE INVENTION

The present invention discloses novel vectors, which meet the requirements of a carrier vector of a gene or genes of interest or of an optimal DNA vaccination vector and which are preferably devoid of drawbacks and side effects of prior art vectors.

25 The present invention is based on the surprising finding that a vector (plasmid) carrying (i) an expression cassette of a DNA sequence encoding a nuclear-anchoring protein, and (ii) multiple copies of high affinity binding sites for said nuclear-anchoring protein spreads in proliferating cells. As a result, the number of vector-carrying cells increases even without the replication of the vector.
30 When the vector additionally carries a gene or genes of interest, the number of such cells that express a gene or genes of interest similarly increases without the replication of the vector. Thus, the vector of the invention lacks a papilloma

virus origin of replication. In a preferred embodiment, the vector of the invention lacks an origin of replication that functions in a mammalian cell.

Accordingly, the present invention discloses novel vectors useful as carrier vectors of a gene or genes of interest, in DNA vaccination and gene therapy and 5 as gene therapeutic agents. In a specific embodiment, said vectors are capable of spreading and, if desired, of expressing a gene or genes of interest in an increasing number of cells for an extended time. The vectors of the present invention preferably express only a nuclear-anchoring protein, and, if desired, the gene or genes of interest, and optionally a selectable marker. However, they preferably lack any redundant, oncogenically transforming or potentially toxic sequences, thereby avoiding a severe drawback of the vectors previously disclosed or suggested for use as DNA vaccines, i.e. hypersensitivity reactions against other viral components. In certain embodiments of the invention, this is achieved by low level of the expressed nuclear-anchoring protein in the cells. At the same time, the 10 vectors of the present invention induce both humoral and cellular immune responses, where the gene or genes of interest is included in the vector.

15

The vectors of the present invention are advantageous for use both in vitro (e.g., in the production level) and in vivo (e.g., vaccination).

The present invention relates to the subject matter of the invention as set 20 forth in the attached claims.

The present invention relates to expression vectors comprising: (a) a DNA sequence encoding a nuclear-anchoring protein operatively linked to a heterologous promoter, said nuclear-anchoring protein comprising (i) a DNA binding domain which binds to a specific DNA sequence, and (ii) a functional domain that 25 binds to a nuclear component, or a functional equivalent thereof; and (b) a multimerized DNA sequence forming a binding site for the nuclear anchoring protein, wherein said vector lacks a papilloma virus origin of replication. In a preferred embodiment a vector of the invention lacks an origin of replication functional in a mammalian cell.

In certain embodiments, the nuclear component is mitotic chromatin, the 30 nuclear matrix, nuclear domain 10 (ND10), or nuclear domain POD.

In certain specific embodiments, the nuclear anchoring-protein is a chromatin-anchoring protein, and said functional domain binds mitotic chromatin.

In certain embodiments, the nuclear-anchoring protein contains a hinge or linker region.

5 In certain embodiments, the nuclear-anchoring protein is a natural protein of eukaryotic, prokaryotic, or viral origin. In certain specific embodiments, the natural protein is of viral origin.

In certain embodiments, the nuclear-anchoring protein is a natural protein of eukaryotic origin.

10 In certain embodiments, the nuclear-anchoring protein is that of a papilloma virus or an Epstein-Barr virus.

In specific embodiments, the nuclear-anchoring protein is the E2 protein of Bovine Papilloma Virus type 1 or Epstein-Barr Virus Nuclear Antigen 1.

15 In a specific embodiment, the nuclear-anchoring protein is the E2 protein of Bovine Papilloma Virus type 1.

In specific embodiments, the nuclear-anchoring protein is a High Mobility Group protein.

In certain embodiments, the nuclear-anchoring protein is a non-natural protein.

20 In certain embodiments, the nuclear-anchoring protein is a recombinant protein, a fusion protein, or a protein obtained by molecular modeling techniques.

In specific embodiments, the recombinant protein, fusion protein, or protein obtained by molecular modeling techniques contains any combination of a DNA binding domain which binds to said specific DNA sequence and a functional domain which binds to a nuclear component, wherein said functional domain which binds to a nuclear component is that of a papilloma virus, an Epstein-Barr-Virus, or a High Mobility Group protein.

30 In certain specific embodiments, the recombinant protein, fusion protein, or protein obtained by molecular modeling techniques contains any combination of a DNA binding domain which binds to said specific DNA sequence and a functional domain which binds to a nuclear component, wherein said functional domain which binds to a nuclear component is that of E2 protein of Bovine Papil-

Ioma Virus type 1, Epstein-Barr Virus Nuclear Antigen 1, or a High Mobility Group protein.

In certain embodiments, the vector further comprises one or more expression cassettes of a DNA sequence of interest.

5 In certain embodiments, the DNA sequence of interest is that of an infectious pathogen. In certain embodiments, the infectious pathogen is a virus. In certain specific embodiments, the virus is selected from the group consisting of Human Immunodeficiency Virus (HIV), Herpex Simplex Virus (HSV), Hepatitis C Virus, Influenzae Virus, and Enterovirus.

10 In certain embodiments, the DNA sequence of interest is that of a bacterium. In certain embodiments, the bacterium is selected from the group consisting of Chlamydia trachomatis, Mycobacterium tuberculosis, and Mycoplasma pneumonia. In a specific embodiment, the bacterium is Salmonella.

15 In certain embodiments, the DNA sequence of interest is that of a fungal pathogen. In certain embodiments, the fungal pathogen is Candida albicans.

In certain embodiments, the DNA sequence of interest is of HIV origin.

20 In specific embodiments, the DNA sequence of interest encodes a non-structural regulatory protein of HIV. In more specific embodiments, the non-structural regulatory protein of HIV is Nef, Tat and/or Rev. In a specific embodiment, the non-structural regulatory protein of HIV is Nef.

In certain embodiments, the DNA sequence of interest encodes a structural protein of HIV. In a specific embodiment, the DNA sequence of interest is the gene encoding HIV gp120/gp160.

25 In certain embodiments, the vector of the invention comprises a first expression cassette comprising a DNA sequence of interest which encodes Nef, Tat and/or Rev, and a second expression cassette comprising a DNA sequence of interest which encodes Nef, Tat and/or Rev.

30 In certain embodiments, the vector of the invention comprises a first expression cassette comprising a DNA sequence of interest which encodes Nef, Tat and/or Rev, and a second expression cassette comprising a DNA sequence of interest which encodes a structural protein of HIV.

In certain embodiments, the DNA sequence of interest encodes a protein associated with cancer.

5 In certain embodiments, the DNA sequence of interest encodes a protein associated with immune maturation, regulation of immune responses, or regulation of autoimmune responses. In a specific embodiment, the protein is APECED.

In a specific embodiment, the DNA sequence of interest is the Aire gene.

In certain embodiments, the DNA sequence of interest encodes a protein that is defective in any hereditary single gene disease.

10 In certain embodiments, the DNA sequence of interest encodes a macromolecular drug.

15 In certain embodiments, the DNA sequence of interest encodes a cytokine. In certain specific embodiments, the cytokine is an interleukin selected from the group consisting of IL1, IL2, IL4, IL6 and IL12. In certain other specific embodiments, the DNA sequence of interest encodes an interferon.

20 In certain embodiments, the DNA sequence of interest encodes a biologically active RNA molecule. In certain specific embodiments, the biologically active RNA molecule is selected from the group consisting of inhibitory antisense and ribozyme molecules. In certain specific embodiments, the inhibitory antisense or ribozyme molecules antagonize the function of an oncogene.

A vector of the invention is suitable for the use for the production of a therapeutic macromolecular agent *in vivo*.

In certain embodiments, the invention provides a vector for use as a medicament.

25 In certain embodiments, the invention provides a vector for use as a carrier vector for a gene, genes, or a DNA sequence or DNA sequences of interest, such as a gene, genes, or a DNA sequence or DNA sequences encoding a protein or peptide of an infectious agent, a therapeutic agent, a macromolecular drug, or any combination thereof.

30 In certain specific embodiments, the invention provides a vector for use as a medicament for treating inherited or acquired genetic defects.

In certain embodiments, the invention provides a vector for use as a therapeutic DNA vaccine against an infectious agent.

In certain embodiments, the invention provides a vector for use as a therapeutic agent.

5 The invention further relates to methods for providing a protein to a subject, said method comprising administering to the subject a vector of the invention, wherein said vector (i) further comprises a second DNA sequence encoding the protein to be provided to the subject, which second DNA sequence is operably linked to a second promoter, and (ii) does not encode Bovine Papilloma Virus protein E1, and wherein said subject does not express Bovine Papilloma Virus protein E1.

10 15 The invention further relates to methods for inducing an immune response to a protein in a subject, said method comprising administering to the subject a vector of the invention wherein said vector (i) further comprises a second DNA sequence encoding said protein, which second DNA sequence is operably linked to a second promoter, and (ii) does not encode Bovine Papilloma Virus protein E1, and wherein said subject does not express Bovine Papilloma Virus protein E1.

20 The invention further relates to methods for treating an infectious disease in a subject in need of said treatment, said method comprising administering to said subject a therapeutically effective amount of a vector of the invention, wherein the DNA sequence of interest encodes a protein comprising an immunogenic epitope of an infectious agent.

25 The invention further relates to methods for treating an inherited or acquired genetic defect in a subject in need of said treatment, said method comprising: administering to said subject a therapeutically effective amount of a vector of the invention, wherein said DNA sequence of interest encodes a protein which is affected by said inherited or acquired genetic defect.

30 The invention further relates to methods for expressing a DNA sequence in a subject, said method comprising administering a vector of the invention to said subject.

The invention further relates to methods for expressing a DNA sequence in a subject, treating an inherited or acquired genetic defect, treating an infectious disease, inducing an immune-response to a protein, and providing a protein to a subject, wherein the vector of the invention does not encode Bovine Papilloma Virus protein E1, and wherein said subject does not express Bovine Papilloma Virus protein E1.

In certain embodiments, a vector of the invention is used for production of a protein encoded by said DNA sequence of interest in a cell or an organism.

The invention further provides a method for the preparation of a vector of claim 1, 2, or 17 comprising: (a) cultivating a host cell containing said vector and (b) recovering the vector. In a specific embodiment, the method for preparing a vector of the invention further comprises before step (a) a step of transforming said host cell with said vector. In certain specific embodiments, the host cell is a prokaryotic cell. In a specific embodiment, the host cell is an Escherichia coli.

The invention further relates to a host cell that is characterized by containing a vector of the invention. In certain embodiments, the host cell is a bacterial cell. In a certain other embodiments, the host cell is a mammalian cell.

The invention further relates to carrier vectors containing a vector of the invention.

The invention further relates to a pharmaceutical composition comprising a vector of the invention and a suitable pharmaceutical vehicle.

The invention further relates to a DNA vaccine containing a vector of the invention.

The invention further relates to a gene therapeutic agent containing a vector of the invention.

The invention further relates to a method for the preparation of a DNA vaccine, said method comprising combining a vector of the invention with a suitable pharmaceutical vehicle.

The invention further relates to a method for the preparation of an agent for use in gene therapy, said method comprising combining a vector of the invention with a suitable pharmaceutical vehicle.

4. DESCRIPTION OF THE FIGURES

Figure 1 shows the schematic map of plasmid super6.

Figure 2 shows the schematic map of plasmid VI.

Figure 3 shows the schematic map of plasmid II.

5 Figure 4 shows the expression of the Nef and E2 proteins from the vectors super6, super6wt, VI, VIwt, and II in Jurkat cells.

Figure 5 shows the schematic map of plasmid product1.

Figure 6A shows the schematic map of the plasmids NNV-1 and NNV-2 and Figure 6B shows the schematic map of plasmid and NNV-2wt.

10 Figure 7 shows the expression of the Nef protein from the plasmids NNV-1, NNV-2, NNV-1wt, NNV-2-wt, super6, and super6wt in Jurkat cells.

Figure 8 shows the expression of the Nef and E2 proteins from the plasmids NNV-2-wt, NNV-2-wtFS, and product I in Jurkat cells.

15 Figure 9 shows the expression of the Nef and E2 proteins from the plasmids NNV-2-wt, NNV-2-wtFS, and product I in P815 cells.

Figure 10 shows the expression of the Nef and E2 proteins from the plasmids NNV-2-wt, NNV-2-wtFS, and product I in CHO cells.

Figure 11 shows the expression of the Nef protein from the plasmids NNV-2-wt, NNV-2-wtFS, and product I in RD cells.

20 Figure 12 shows the expression of the RNA molecules NNV-2wt in CHO, Jurkat cells, and P815 cells.

Figure 13 shows the stability of NNV-2wt in bacterial cells.

Figure 14 shows the Southern blot analysis of stability of the NNV-2wt as non-replicating episomal element in CHO and Jurkat cell lines.

25 Figure 15 shows that the vectors NNV2wt, NNV2wtFS and product1 are unable to HPV-11 replication factor-dependent replication.

Figure 16 shows the schematic map of the plasmid 2wtd1EGFP.

Figure 17 shows the schematic map of the plasmid gf10bse2

Figure 18 shows the schematic map of the plasmid 2wtd1EGFPFS.

30 Figure 19 shows the schematic map of the plasmid NNVd1EGFP.

Figure 20 shows the growth curves of the Jurkat cells transfected with the plasmids 2wtd1EGFP, 2wtd1EGFPFS, NNVd1EGFP or with carrier DNA only.

Figure 21 shows the growth curves of the Jurkat cells transfected with the plasmids 2wtd1EGFP, 2wtd1EGFPFS, gf10bse2 or with carrier DNA only.

Figure 22 shows the change in the percentage of d1EGFP positive cells in a population of Jurkat cells transfected with the vectors 2wtd1EGFP, 5 2wtd1EGFPFS or NNVd1EGFP.

Figure 23 shows the change in percentage of the d1EGFP positive cells in a population of Jurkat cells transfected with the vectors 2wtd1EGFP, 2wtd1EGFPFS or gf10bse2.

Figure 24 shows the change in the number of d1EGFP expressing cells in 10 a population of Jurkat cells transfected with the vectors 2wtd1EGFP, 2wtd1EGFPFS or NNVd1EGFP.

Figure 25 shows the change in the number of d1EGFP expressing cells in a population of Jurkat cells transfected with the vectors 2wtd1EGFP, 2wtd1EGFPFS or gf10bse2.

15 Figure 26. T-cell responses towards recombinant Nef proteins (5 micrograms/well), measured by T-cell proliferation in five patients immunized with 1 microgram of GTU-Nef.

Figure 27. T-cell responses towards recombinant Nef proteins (5 micrograms/well), measured by T-cell proliferation in five patients immunized with 20 20 micrograms of GTU-Nef.

Figure 28. T-cell responses towards recombinant Nef proteins (5 micrograms/well), measured by T-cell proliferation in patient # 1 immunized with 1 microgram of GTU-Nef. The results are given as stimulation index of the T-cell proliferation assay (Nef SI) and as IFN-Gamma secretion to the supernatant.

25 Figure 29. (A) plasmid pEBO LPP; (B) plasmid s6E2d1EGFP; (C) plasmid FRE2d1EGFP

Figure 30. Plasmid FREBNAd1EGFP

Figure 31. Vectors did not interfere with cell proliferation

Figure 32. Vectors were maintained in the cells with different kinetics

30 Figure 33. Change of the number of d1EGFP expressing cells in time in transfected total population of cells

Figure 34. Change of the number of d1EGFP expressing cells in time in transfected total population of cells. (A) human embryonic cell line 293; (B) mouse cell line 3T6

Figure 35. Nef and E2 antibody response

5 Figure 36. Rev and Tat antibody response

Figure 37. Gag and CTL response

Figure 38. (A) GTU-1; (B) GTU-2Nef; (C) GTU-3Nef; (D) super6wtd1EGFP; (E) FREBNAAd1EGFP; (F) E2BSEBNAd1EGFP; (G) NNV-Rev

10 Figure 39. (A) pNRT; (B) pTRN; (C) pRTN; (D) pTNR; (E) pRNT; (F) p2TRN; (G) p2RNT; (H) p3RNT; (I) pTRN-iE2-GMCSF; (J) pTRN-iMG-GMCSF

Figure 40. (A) pMV1NTR; (B) pMV2NTR; (C) pMV1N11TR; (D) pMV2N11TR

Figure 41. (A) pCTL; (B) pdgag; (C) psynp17/24; (D) poptp17/24; (E) p2mCTL; (F) p2optp17/24; (G) p3mCTL; (H) p3optp17/24

15 Figure 42. (A) pTRN-CTL; (B) pRNT-CTL; (C) pTRN-dgag; (D) pTRN-CTL-dgag; (E) pRNT-CTL-dgag; (F) pTRN-dgag-CTL; (G) pRNT-dgag-CTL; (H) pTRN-optp17/24-CTL; (I) pTRN-CTL-optp17/24; (J) pRNT-CTL-optp17/24; (K) p2TRN-optp17/24-CTL; (L) p2RNT-optp17/24-CTL; (M) p2TRN-CTL-optp17/24; (N) p2RNT-CTL-optp17/24; (O) p2TRN-CTL-optp17/24-iE2-mGMCSF; (P) p2RNT-CTL-optp17/24-iE2-mGMCSF; (Q) p3TRN-CTL-optp17/24; (R) p3RNT-CTL-optp17/24; (S) p3TRN-CTL-optp17/24-iE2-mGMCSF; (T) p3RNT-CTL-optp17/24-iE2-mGMCSF; (U) FREBNA-RNT-CTL-optp17/24; (V) super6wt-RNT-CTL-optp17/24; (W) E2BSEBNA- RNT-CTL-optp17/24; (X) pCMV- RNT-CTL-optp17/24

25 Figure 43. Analysis of expression of the multireg antigens.

Figure 44. Analysis of expression of the multireg antigens comprised of immunodominant parts of the proteins.

Figure 45. Analysis of intracellular localization of multireg antigens by immunofluorescence.

30 Figure 46. Analysis of expression of the gag coded structural proteins and the CTL multi-epitope.

47. The p17/24 protein localization in membranes of RD cells.

Figure 48. Analysis expression of the dgag and CTL containing multi-genes in Cos-7 cells.

Figure 49. Western blot analyses of multiHIV antigens expressed in Jurkat cells.

5 Figure 50. Analysis of the expression of the TRN-CTL-optp17/24 and RNT-CTL-optp17/24 antigens as well E2 protein from the GTU-1, GTU-2 and GTU-3 vector.

Figure 51. The maintenance of the multiHIV antigen expression from different vectors.

10 Figure 52. Intracellular localization of the multiHIV antigens in RD cells.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1 VECTORS OF THE INVENTION

The present invention is based on the unexpected finding that expression vectors, which carry (A) an expression cassette of a gene of a nuclear-anchoring protein that binds both to (i) a specific DNA sequence and (ii) to a suitable nuclear component and (B) a multimerized DNA binding sequence for said nuclear-anchoring protein are capable of spreading in a proliferating cell population. Such nuclear-anchoring proteins include, but are not limited to, chromatin-anchoring proteins, such as the Bovine Papilloma Virus type 1 E2 protein (BPV1 E2; SEQ ID NO: 50). The DNA binding sequences can be, but are not limited to, multimerized E2 binding sites. On the basis of prior art, it could not be expected that a segregation/partitioning function of, for instance, the papilloma viruses could be expressed separately and that an addition of such segregation/partitioning function to the vaccine vectors would assure the distribution of the vector in the proliferating cell population. Additionally, on the basis of the prior art, it could not have been expected that functional vectors acting independently of the replication origin can be constructed.

30 The term "nuclear-anchoring protein" as used in the present invention refers to a protein, which binds to a specific DNA sequence and capable of providing a nuclear compartmentalization function to the vector, i.e., to a protein, which is capable of anchoring or attaching the vector to a specific nuclear compartment.

- In certain embodiments of the invention, the nuclear-anchoring protein is a natural protein. Examples of such nuclear compartments are the mitotic chromatin or mitotic chromosomes, the nuclear matrix, nuclear domains like ND10 and POD etc. Examples of nuclear-anchoring proteins are the Bovine Papilloma Virus type 5 1 (BPV1) E2 protein, EBNA1 (Epstein-Barr Virus Nuclear Antigen 1; SEQ ID NO: 52), and High Mobility Group (HMG) proteins etc. The term "functional equivalent of a nuclear-anchoring protein" as used in the present invention refers to a protein or a polypeptide of natural or non-natural origin having the properties of the nuclear-anchoring protein.
- 10 In certain other embodiments of the invention, the nuclear-anchoring protein of the invention is a recombinant protein. In certain specific embodiments of the invention, the nuclear-anchoring protein is a fusion protein, a chimeric protein, or a protein obtained by molecular modeling. A fusion protein, or a protein obtained by molecular modeling in connection with the present invention is characterized by its ability to bind to a nuclear component and by its ability to bind sequence-specifically to DNA. In a preferred embodiment of the invention, such a fusion protein is encoded by a vector of the invention which also contains the specific DNA sequence to which the fusion/chimeric protein binds. Nuclear components include, but are not limited to chromatin, the nuclear matrix, the ND10 domain and POD. In order to reduce the risk of interference with the expression of genes endogenous to the host cell, the DNA binding domain and the corresponding DNA sequence is preferably non-endogenous to the host cell/host organism. Such domains include, but are not limited to, the DNA binding domain of the Bovine Papilloma Virus type 1 (BPV1) E2 protein (SEQ ID NO: 50), Epstein-Barr Virus Nuclear Antigen 1 (EBNA1; SEQ ID NO: 52), and High Mobility Group 15 20 25 (HMG) proteins (HMG box).

The vector of the invention can further comprise a "DNA sequence of interest", that encodes a protein (including a peptide or polypeptide), e.g., that is an immunogen or a therapeutic. In certain embodiments of the invention, the DNA sequence of interest encodes a biologically active RNA molecule, such as an antisense RNA molecule or a ribozyme.

The expression vectors of the invention carrying an expression cassette for a gene of a nuclear-anchoring protein and multimerized binding sites for said nuclear-anchoring protein spread in a proliferating host cell population. This means that a high copy-number of vectors or plasmids are delivered into the target cells 5 and the use of the segregation/partitioning function of the nuclear-anchoring protein and its multimerized binding sites assures the distribution of the vector to the daughter cells during cell division.

The vector of the invention lacks a papilloma virus origin of replication. Further, in a preferred embodiment, the vector of the invention lacks an origin of 10 replication functional in a mammalian cell. The omission of a papilloma virus origin of replication or a mammalian origin of replication constitutes an improvement over prior art vectors for several reasons. (1) Omission of the origin of replication reduces the size of the vector of the invention compared to prior art vectors. Such a reduction in size increases the stability of the vector and facilitates uptake 15 by the host cell. (2) Omission of the origin of replication reduces the risk for recombination with the host cell's genome, thereby reducing the risk of unwanted side effects. (3) The omission of the origin of replication allows to control the dosage simply by adjusting the amount of vector administered. In contrast, with a functioning origin of replication, replication of the vector has to be taken into consideration when determining the required dosage. (4) If the vector is not administered 20 to a host organism continually, the lack of an origin of replication allows the host organism to clear itself of the vector, thus providing more control over the levels of DNA sequences to be expressed in the host organism. Further, the ability of the organism to clear itself of the vector will be advantageous if the presence 25 of the vector is required only during the course of a therapy but is undesirable in a healthy individual.

The gene of a nuclear-anchoring protein useful in the vectors of the present invention can be any suitable DNA sequence encoding a natural or artificial protein, such as a recombinant protein, a fusion protein or a protein obtained by 30 molecular modeling techniques, having the required properties. Thus the gene of a natural nuclear-anchoring protein, which contains a DNA binding domain capable of binding to a specific DNA sequence and a functional domain capable of

binding to a nuclear component, can be that of a viral protein, such as the E2 protein of Bovine Papilloma Virus or the EBNA1 (Epstein-Barr Virus Nuclear Antigen 1) of the Epstein-Barr Virus, a eukaryotic protein such as one of the High Mobility Group (HMG) proteins or a like protein, or a prokaryotic protein. Alternatively, the 5 gene of a nuclear-anchoring protein, which contains a DNA binding domain capable of binding to a specific DNA sequence and a functional domain capable of binding to a nuclear component, can also be comprised of DNA sequences, which encode a domain from a cellular protein having the ability to attach to a suitable nuclear structure, such as to mitotic chromosomes, the nuclear matrix or 10 nuclear domains like ND10 or POD.

Alternatively, the DNA sequence, which encodes a non-natural or artificial protein, such as a recombinant protein or a fusion protein or a protein obtained by molecular modeling, which contains a DNA binding domain capable of binding to a specific DNA sequence of, e.g., a papilloma virus, such as the DNA binding 15 domain of the E2 protein of the BPV1, but in which the N-terminus of the nuclear-anchoring protein, e.g. that of the E2 protein, has been replaced with domains of any suitable protein of similar capacity, for example, with the N-terminal domain of Epstein-Barr Virus Nuclear Antigen 1 sequence, can be used. Similarly, DNA sequences, which encode a recombinant protein or a fusion protein, which contains a functional domain capable of binding to a nuclear component, e.g., the N- 20 terminal functional domain of a papilloma virus, such as the E2 protein of the BPV1, but in which the C-terminal DNA-binding dimerization domain of the nuclear-anchoring protein, e.g., that of the E2 protein, has been replaced with domains of any protein of a sufficient DNA-binding strength, e.g., the DNA binding 25 domain of the BPV-1 E2 protein and the EBNA-1, can be used.

In a preferred embodiment of the invention, the nuclear-anchoring protein is a chromatin-anchoring protein, which contains a DNA binding domain, which binds to a specific DNA sequence, and a functional domain capable of binding to mitotic chromatin. A preferred example of such a chromatin-anchoring protein 30 and its multimerized binding sites useful in the present invention are the E2 protein of Bovine Papilloma Virus type 1 and E2 protein multimerized binding sites. In the case of E2, the mechanism of the spreading function is due to the dual

function of the E2 protein: the capacity of the E2 protein to attach to mitotic chromosomes through the N-terminal domain of the protein and the sequence-specific binding capacity of the C-terminal domain of the E2 protein, which assures the tethering of vectors, which contain a multimerized E2 binding site, to mitotic 5 chromosomes. A segregation/partitioning function is thus provided to the vectors.

In another preferred embodiment of the invention, the expression cassette of a gene of the chromatin-anchoring protein comprises a gene of any suitable protein of cellular, viral or recombinant origin having analogous properties to E2 of the BPV1, i.e., the ability to attach to the mitotic chromatin through one domain 10 and to cooperatively bind DNA through another domain to multimerized binding sites specific for this DNA binding domain.

In a specific embodiment, sequences obtained from BPV1, are used in the vectors of the present invention, they are extensively shortened in size to include just two elements from BPV1. First, they include the E2 protein coding sequence 15 transcribed from a heterologous eukaryotic promoter and polyadenylated at the heterologous polyadenylation site. Second, they include E2 protein multiple binding sites incorporated into the vector as a cluster, where the sites can be as head-to-tail structures or can be included into the vector by spaced positioning. Both of these elements are necessary and, surprisingly, sufficient for the function of the 20 vectors to spread in proliferating cells. Similarly, when DNA sequences based of other suitable sources are used in the vectors of the present invention, the same principles are applied.

According to the present invention, the expression cassette of a gene of a nuclear-anchoring protein, which contains a DNA binding domain capable of binding 25 to a specific DNA sequence and a functional domain capable of binding to a nuclear component, such as an expression cassette of a gene of a chromatin-anchoring protein, like BPV1 E2, comprises a heterologous eukaryotic promoter, the nuclear-anchoring protein coding sequence, such as a chromatin-anchoring protein coding sequence, for instance the BPV1 E2 protein coding sequence, and 30 a poly A site. Different heterologous, eukaryotic promoters, which control the expression of the nuclear-anchoring protein, can be used. Nucleotide sequences of such heterologous, eukaryotic promoters are well known in the art and are readily

available. Such heterologous eukaryotic promoters are of different strength and tissue-specificity. In a preferred embodiment, the nuclear anchoring protein is expressed at low levels.

The multimerized DNA binding sequences, i.e., DNA sequences containing 5 multimeric binding sites, as defined in the context of the present invention, are the region, to which the DNA binding dimerization domain binds. The multimerized DNA binding sequences of the vectors of the present invention can contain any suitable DNA binding site, provided that it fulfills the above requirements.

In a preferred embodiment, the multimerized DNA binding sequence of a 10 vector of the present invention can contain any one of known 17 different affinity E2 binding sites as a hexamer or a higher oligomer, as a octamer or a higher oligomer, as a decamer or higher oligomer. Oligomers containing different E2 binding sites are also applicable. Specifically preferred E2 binding sites useful in the 15 vectors of the present invention are the BPV1 high affinity sites 9 and 10, affinity site 9 being most preferred. When a higher oligomer is concerned, its size is limited only by the construction circumstances and it may contain from 6 to 30 identical binding sites. Preferred vectors of the invention contain 10 BPV-1 E2 binding sites 9 in tandem. When the multimerized DNA binding sequences are comprised 20 of different E2 binding sites, their size and composition is limited only by the method of construction practice. Thus they may contain two or more different E2 binding sites attached to a series of 6 to 30, most preferably 10, E2 binding sites. The Bovine Papilloma Virus type 1 genome (SEQ ID NO: 49) contains 17 E2 protein binding sites which differ in their affinity to E2. The E2 binding sites are described in Li et al. [Genes Dev 3(4) (1989) 510-526], which is incorporated by 25 reference in its entirety herein.

Alternatively, the multimerized DNA binding sequences may be composed of any suitable multimeric specific sequences capable of inducing the cooperative binding of the protein to the plasmid, such as those of the EBNA1 or a suitable HMG protein. 21x30bp repeats of binding sites for EBNA-1 are localized in the 30 region spanning from nucleotide position 7421 to nucleotide position 8042 of the Epstein-Barr virus genome (SEQ ID NO:51). These EBNA-1 binding sites are described in the following references: Rawlins et al., Cell 42(3) (1985) 859-868;

Reisman et al., Mol Cell Biol 5(8) (1985) 1822-1832; and Lupton and Levine, Mol Cell Biol 5(10) (1985) 2533-2542, all three of which are incorporated by reference in their entireties herein.

The position of the multimerized DNA binding sequences relative to the expression cassette for the DNA binding dimerization domain is not critical and can be any position in the plasmid. Thus the multimerized DNA binding sequences can be positioned either downstream or upstream relative to the expression cassette for the gene of interest, a position close to the promoter of the gene of interest being preferred.

10 The vectors of the invention also contain, where appropriate, a suitable promoter for the transcription of the gene or genes or the DNA sequences of interest, additional regulatory sequences, polyadenylation sequences and introns. Preferably the vectors may also include a bacterial plasmid origin of replication and one or more genes for selectable markers to facilitate the preparation of the
15 vector in a bacterial host and a suitable promoter for the expression the gene for antibiotic selection.

20 The selectable marker can be any suitable marker allowable in DNA vaccines, such a kanamycin or neomycin, and others. In addition, other positive and negative selection markers can be included in the vectors of the invention, where applicable.

25 The vectors of the present invention only comprise the DNA sequences, for instance BPV1 DNA sequences, which are necessary and sufficient for long-term maintenance. All superfluous sequences, which may induce adverse reactions, such as oncogenic sequences, have been deleted. Thus in preferred vectors of the invention the E2 coding sequence is modified by mutational analysis so that this expresses only E2 protein and overlapping E3, E4 and E5 sequences have been inactivated by the introduction of mutations, which deactivate the translation from Open Reading Frames for E3, E4 and E5. The vector of the invention does not contain a papilloma virus origin of replication. Preferably, the vector of
30 the invention further does not contain an origin of replication functional in a mammalian cell or a mammal.

Furthermore, the vectors of the present invention are not host specific, since the expression of the nuclear-anchoring protein, such as the E2 protein, is controlled by non-native or heterologous promoters. Depending on the particular promoter chosen, these promoters may be functional in a broad range of mammalian cells or they can be cell or tissue specific. Examples of promoters for the nuclear-anchoring protein, such as for the E2 protein, useful in the vectors of the present invention are thymidine kinase promoters, Human Cytomegalovirus Immediate Early Promoter, Rous Sarcoma Virus LTR and like. For the expression of the gene of interest, preferred promoters are strong promoters assuring high levels of expression of the gene of interest, an example for such a promoter is the Human Cytomegalovirus Immediate Early Promoter.

5.2 THE VECTORS OF THE INVENTION AS VEHICLES FOR EXPRESSION OF A DNA SEQUENCE OF INTEREST

A gene, genes or a DNA sequence or DNA sequences to be expressed via a vector of the invention can be any DNA sequence of interest, whose expression is desired. Thus the vectors may contain a gene or genes or a DNA sequence or DNA sequences from infectious microbial pathogens, such as viruses, against which live attenuated vaccines or inactivated vaccines cannot be prepared or used. Such DNA sequences of interest include genes or DNA sequences from viruses, such as Human Immunodeficiency Virus (HIV), Herpex Simplex Virus (HSV), Hepatitis C Virus, Influenzae Virus, Enteroviruses etc.; intracellular bacterial, such as Chlamydia trachomatis, Mycobacterium tuberculosis, Mycoplasma pneumonia etc.; extracellular bacteria, such as Salmonella; or fungi, such as Candida albicans.

In a preferred embodiment of the invention, the vectors contain a gene encoding early regulatory proteins of HIV, i.e. the nonstructural regulatory proteins Nef, Tat or Rev, preferably Nef. In another preferred embodiment of the invention the vectors of the invention contain genes encoding structural proteins of the HIV. In another preferred embodiment the vectors of the present invention contain two or more genes encoding any combination of early regulatory proteins and/or structural proteins of HIV. Illustrative examples of such combinations are a com-

bination of a gene encoding the Nef protein and a DNA sequence encoding the Tat protein, possibly together with a DNA sequence encoding outer envelope glycoprotein of HIV, gp120/gp160 or a combination of any immunogenic epitopes of the proteins of pathogens incorporated into artificial recombinant protein.

5 Alternatively, the vectors of the invention may contain genes or DNA sequences for inherited or acquired genetic defects, such as sequences of differentiation antigens for melanoma, like a Tyrosinase A coding sequence or a coding sequence of beta-catenins.

10 In a preferred embodiment of the invention, the vectors contain a gene encoding proteins relating to cancer or other mutational diseases, preferably diseases related to immune maturation and regulation of immune response towards self and nonself, such as the APECED gene.

15 In another preferred embodiment of the invention, the vectors contain any DNA sequence coding for a protein that is defective in any hereditary single gene hereditary disease.

In another preferred embodiment of the invention, the vectors contain any DNA sequence coding for a macromolecular drug to be delivered and produced in vivo.

20 The method of the invention for the preparation of the vectors of the invention comprises the following steps: (A) cultivating a host cell containing a vector of the invention, and (B) recovering the vector. In certain specific embodiments, step (A) is preceded by transforming a host cell with a vector of the invention.

25 The vectors of the invention are preferably amplified in a suitable bacterial host cell, such as Escherichia coli. The vectors of the invention are stable and replicate at high copy numbers in bacterial cells. If a vector of the invention is to be amplified in a bacterial host cell, the vector of the invention contains a bacterial origin of replication. Nucleotide sequences of bacterial origins of replication are well known to the skilled artisan and can readily be obtained.

30 Upon transfection into a mammalian host in high copy number, the vector spreads along with cell divisions and the number of cells carrying the vector increases without the replication of the vector, each cell being capable of expressing the protein of interest.

The vectors of the invention result in high expression of the desired protein. For instance, as demonstrated in Examples 4, 7 - 10: a high expression of the Nef protein of the HIV, green fluorescent protein (EGFP) and the AIRE protein could be demonstrated in many different cell lines and the data indicate that not only the number of positive cells, but the quantity of the protein encoded by the gene of interest is increasing in time.

The vectors of the invention also induce both humoral and cellular response as demonstrated in Examples 9 and 10. The results indicate that the vectors of the present invention can effectively be used as DNA vaccines.

10 The vaccines of the present invention contain a vector of the present invention or a mixture of said vectors in a suitable pharmaceutical carrier. The vaccine may for instance contain a mixture of vectors containing genes for the three different regulatory proteins of the HIV and/or structural proteins of the HIV.

15 The vaccines of the invention are formulated using standard methods of vaccine formulation to produce vaccines to be administered by any conventional route of administration, i.e. intramuscularly, intradermally and like.

The vectors of the invention may contain the ISS stimulatory sequences in order to activate the immune response of the body.

20 The vaccines of the invention can be used in a conventional preventive manner to protect an individual from infections. Alternatively, the vaccines of the invention can be used as therapeutical vaccines, especially in the case of viral infections, together with a conventional medication.

25 As mentioned above, the vectors of the present invention carrying the mechanism of spreading in the host cell find numerous applications as vaccines, in gene therapy, in gene transfer and as therapeutic immunogens. The vectors of the invention can be used to deliver a normal gene to a host having a gene defect, thus leading to a cure or therapy of a genetic disease. Furthermore, the vectors can deliver genes of immunogenic proteins of foreign origin, such as those from microbes or autologous tumor antigens, to be used in the development of 30 vaccines against microbes or cancer. Furthermore, the vectors of the invention can deliver suitable genes of marker substances to nucleus, to be used in studies of cellular function or in diagnostics. Finally, the vectors of the invention can be

used to specifically deliver a gene or macromolecular drug to the nucleus, thus enabling the development of novel therapeutic principles to treat and cure diseases, where the expression of the drug in the site of action, the cell nucleus, is of importance. These drugs can be chemical macromolecules, such as any proteins or polypeptides with therapeutic or curative effect, which interfere with any of the nuclear mechanisms, such as the replication or transcription or the transport of substances to and from the nucleus.

Specifically, the vectors of the present invention can be used for the expression of the specific cytokines, like interleukines (IL1, IL2, IL4, IL6, IL12 and others) or interferon, with the aim of modulating the specific immune responses of the organism (immunotherapy) against foreign antigens or boosting of the activity of the immune system against the mutated self-antigens. The vectors of the present invention are also useful in complementing malfunctioning of the brain due to the loss of specific dopamine-ergic neurons leading to the irreversible neurodegeneration, which is cause for Parkinson's disease, by expressing genes involved into synthesis of dopamine, like tyrosine hydroxylase, as well as other genes deficiency of which would have the similar effect. The vectors of the present invention are also useful for the expression of proteins and peptides regulating the brain activity, like dopamine receptors, CCK-A and CCK-B receptors, as well as neurotrophic factors, like GDNF, BDNF and other proteins regulating the brain activity. Further, the vectors of the present invention are useful for a long-term expression of factor IX in hepatocytes and alfa1-antitrypsin in muscle cells with the aim of complementing respective deficiencies of the organism.

25 5.3 TARGET DISEASES AND DISORDERS

In certain embodiments, a vector of the invention is used as a vaccine. In certain embodiments, a vector of the invention contains a DNA sequence of interest that encodes a protein or a peptide. Upon administering of such a vector to a subject, the protein or peptide encoded by the DNA sequence of interest is expressed and stimulates an immune response specific to the protein or peptide encoded by the DNA sequence of interest.

In specific embodiments, the vector of the invention is used to treat and/or prevent an infectious disease and/or a condition caused by an infectious agent. Such diseases and conditions include, but are not limited to, infectious diseases caused by bacteria, viruses, fungi, protozoa, helminths, and the like. In a more 5 specific embodiment of the invention, the infectious disease is Acquired Immuno-deficiency Syndrome.

Preferably, where it is desired to treat or prevent viral diseases, DNA sequences encoding molecules comprising epitopes of known viruses are used. For example, such DNA sequences encoding antigenic epitopes may be prepared from viruses including, but not limited to, hepatitis type A, hepatitis type B, hepatitis type C, influenza, varicella, adenovirus, herpes simplex type I (HSV-I), 10 herpes simplex type II (HSV-II), rinderpest, rhinovirus, echovirus, rotavirus, respiratory syncytial virus, papilloma virus, papova virus, cytomegalovirus, echinovirus, arbovirus, huntavirus, coxsackie virus, mumps virus, measles virus, rubella virus, 15 polio virus, human immunodeficiency virus type I (HIV-I), and human immunodeficiency virus type II (HIV-II).

Preferably, where it is desired to treat or prevent bacterial infections, DNA sequences encoding molecules comprising epitopes of known bacteria are used. For example, such DNA sequences encoding antigenic epitopes may be prepared from bacteria including, but not limited to, mycobacteria rickettsia, 20 mycoplasma, neisseria and legionella.

Preferably, where it is desired to treat or prevent protozoal infections, DNA sequences encoding molecules comprising epitopes of known protozoa are used. For example, such DNA sequences encoding antigenic epitopes may be prepared from protozoa including, but not limited to, leishmania, kokzidioa, and trypanosoma. 25

Preferably, where it is desired to treat or prevent parasitic infections, DNA sequences encoding molecules comprising epitopes of known parasites are used. For example, such DNA sequences encoding antigenic epitopes may be prepared from parasites including, but not limited to, chlamydia and rickettsia. 30

In other specific embodiments, the vector of the invention is used to treat and/or prevent a neoplastic disease in a subject. In these embodiments, the DNA

sequence of interest encodes a protein or peptide that is specific to or associated with the neoplastic disease. By way of non-limiting example, the neoplastic disease can be a fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, 5 medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, 10 acoustic neuroma, oligodendrogloma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, e.g., acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's 15 disease and non-Hodgkin's disease), multiple myeloma, Waldenström's macroglobulinemia, and heavy chain disease, etc.

In certain other embodiments of the invention, the DNA sequence of interest encodes a protein that is non-functional or malfunctioning due to an inherited disorder or an acquired mutation in the gene encoding the protein. Such genetic diseases include, but are not limited to, metabolic diseases, e.g., Atherosclerosis (affected gene: APOE); cancer, e.g., Familial Adenomatous Polyposis Coli (affected gene: APC gene); auto-immune diseases, e.g., autoimmune polyendocrinopathy-candidosis-ectodermal dysplasia (affected gene: APECED); disorders of the muscle, e.g., Duchenne muscular dystrophy/vaccines (affected gene: DMD); 25 diseases of the nervous system, e.g., Alzheimer's Disease (affected genes: PS1 and PS2).

In even/other embodiments, the vectors of the invention are used to treat and/or prevent diseases and disorders caused by pathologically high activity of a protein. In these embodiments of the invention, the DNA sequence of interest encodes an antagonist of the overactive protein. Such antagonists include, but 5 are not limited to, antisense RNA molecules, ribozymes, antibodies, and dominant negative proteins. In specific embodiments of the invention, the DNA sequence of interest encodes an inhibitor of an oncogene.

In certain embodiments, the DNA sequence of interest encodes a molecule that antagonizes neoplastic growth. In specific embodiments of the invention, the DNA sequence of interest encodes a tumor suppressor, such as, but not 10 limited to, p53. In other specific embodiments, the DNA sequence of interest encodes an activator of apoptosis, such as but not limited to, a Caspase.

The invention provides methods, whereby a DNA sequence of interest is expressed in a subject. In certain embodiments, a vector containing one or more 15 expression cassettes of a DNA sequence of interest is administered to the subject, wherein the subject does not express the Bovine Papilloma Virus E1 protein.

5.4 THERAPEUTIC METHODS FOR USE WITH THE INVENTION

20 5.4.1 RECOMBINANT DNA

In various embodiments of the invention, the vector of the invention comprises one or more expression cassettes comprising a DNA sequence of interest. The DNA sequence of interest can encode a protein and/or a biologically active 25 RNA molecule. In either case, the DNA sequence is inserted into the vector of the invention for expression in recombinant cells or in cells of the host in the case of gene therapy.

An expression cassette, as used herein, refers to a DNA sequence of interest operably linked to one or more regulatory regions or enhancer/promoter sequences which enables expression of the protein of the invention in an appropriate host cell. "Operably-linked" refers to an association in which the regulatory 30 regions and the DNA sequence to be expressed are joined and positioned in such a way as to permit transcription, and in the case of a protein, translation.

The regulatory regions necessary for transcription of the DNA sequence of interest can be provided by the vector of the invention. In a compatible host-construct system, cellular transcriptional factors, such as RNA polymerase, will bind to the regulatory regions of the vector to effect transcription of the DNA sequence of interest in the host organism. The precise nature of the regulatory regions needed for gene expression may vary from host cell to host cell. Generally, a promoter is required which is capable of binding RNA polymerase and promoting the transcription of an operably-associated DNA sequence. Such regulatory regions may include those 5'-non-coding sequences involved with initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like. The non-coding region 3' to the coding sequence may contain transcriptional termination regulatory sequences, such as terminators and polyadenylation sites.

Both constitutive and inducible regulatory regions may be used for expression of the DNA sequence of interest. It may be desirable to use inducible promoters when the conditions optimal for growth of the host cells and the conditions for high level expression of the DNA sequence of interest are different. Examples of useful regulatory regions are provided below (section 5.4.4).

In order to attach DNA sequences with regulatory functions, such as promoters, to the DNA sequence of interest or to insert the DNA sequence of interest into the cloning site of a vector, linkers or adapters providing the appropriate compatible restriction sites may be ligated to the ends of the cDNAs by techniques well known in the art [Wu et al., Methods in Enzymol 152 (1987) 343-349]. Cleavage with a restriction enzyme can be followed by modification to create blunt ends by digesting back or filling in single-stranded DNA termini before ligation. Alternatively, a desired restriction enzyme site can be introduced into a fragment of DNA by amplification of the DNA by use of PCR with primers containing the desired restriction enzyme site.

The vector comprising a DNA sequence of interest operably linked to a regulatory region (enhancer/promoter sequences) can be directly introduced into appropriate host cells for expression of the DNA sequence of interest without further cloning.

For expression of the DNA sequence of interest in mammalian host cells, a variety of regulatory regions can be used, for example, the SV40 early and late promoters, the cytomegalovirus (CMV) immediate early promoter, and the Rous sarcoma virus long terminal repeat (RSV-LTR) promoter. Inducible promoters 5 that may be useful in mammalian cells include but are not limited to those associated with the metallothionein II gene, mouse mammary tumor virus glucocorticoid responsive long terminal repeats (MMTV-LTR), β -interferon gene, and hsp70 gene [Williams et al., Cancer Res. 49 (1989) 2735-42; Taylor et al., Mol. Cell Biol., 10 (1990) 165-75]. It may be advantageous to use heat shock promoters or 10 stress promoters to drive expression of the DNA sequence of interest in recombinant host cells.

In addition, the expression vector may contain a selectable or screenable marker gene for initially isolating, identifying or tracking host cells that contain the vector. A number of selection systems may be used for mammalian cells, including but not limited to the Herpes simplex virus thymidine kinase [Wigler et al., Cell 15 11 (1977) 223], hypoxanthine-guanine phosphoribosyltransferase [Szybalski and Szybalski, Proc. Natl. Acad. Sci. USA 48 (1962) 2026], and adenine phosphoribosyltransferase [Lowy et al., Cell 22 (1980) 817] genes can be employed in tk⁻, hgprt⁻ or aprt⁻ cells, respectively. Also, antimetabolite resistance can be used as 20 the basis of selection for dihydrofolate reductase (dhfr), which confers resistance to methotrexate [Wigler et al., Natl. Acad. Sci. USA 77 (1980) 3567; O'Hare et al., Proc. Natl. Acad. Sci. USA 78 (1981) 1527]; gpt, which confers resistance to mycophenolic acid [Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78 (1981) 2072]; neomycin phosphotransferase (neo), which confers resistance to the aminoglycoside G-418 [Colberre-Garapin et al., J. Mol. Biol. 150 (1981) 1]; and hygromycin 25 phosphotransferase (hgy), which confers resistance to hygromycin [Santerre et al., 1984, Gene 30 (1984) 147]. Other selectable markers, such as but not limited to histidinol and Zeocin® can also be used.

30 5.4.2 EXPRESSION SYSTEMS AND HOST CELLS

For use with the methods of the invention, the host cell and/or the host organism preferably does not express the Bovine Papilloma Virus E1 protein. Fur-

thermore, preferably the vector of the invention does not encode the Bovine Papilloma Virus E1 protein.

Preferred mammalian host cells include but are not limited to those derived from humans, monkeys and rodents, (see, for example, Kriegler M. in, "Gene Transfer and Expression: A Laboratory Manual", New York, Freeman & Co. 1990), such as monkey kidney cell line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293, 293-EBNA, or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen. Virol., 36 (1977) 59; baby hamster kidney cells (BHK, ATCC CCL 10); chinese hamster ovary-cells-DHFR [CHO, Urlaub and Chasin. Proc. Natl. Acad. Sci. 77 (1980) 4216]; mouse sertoli cells [Mather, Biol. Reprod. 23 (1980) 243-251]; mouse fibroblast cells (NIH-3T3), monkey kidney cells (CVI ATCC CCL 70); african green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor cells (MMT 060562, ATCC CCL51).

The vectors of the invention may be synthesized and assembled from known DNA sequences by well-known techniques in the art. The regulatory regions and enhancer elements can be of a variety of origins, both natural and synthetic. Some host cells may be obtained commercially.

The vectors of the invention containing a DNA sequence of interest can be introduced into the host cell by a variety of techniques known in the art, including but not limited to, for prokaryotic cells, bacterial transformation (Hanahan, 1985, in DNA Cloning, A Practical Approach, 1:109-136), and for eukaryotic cells, calcium phosphate mediated transfection [Wigler et al., Cell 11 (1977) 223-232], liposome-mediated transfection [Schaefer-Ridder et al., Science 215 (1982) 166-168], electroporation [Wolff et al., Proc Natl Acad Sci 84 (1987)3344], and micro-injection [Cappechi, Cell 22 (1980) 479-4889].

In a specific embodiment, cell lines that express the DNA sequence of the invention may be engineered by using a vector that contains a selectable marker. By way of example but not limitation, following the introduction of the vector, en-

gineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the vector confers resistance to the selection and optimally allows only cells that contain the vector with the selectable marker to grow in culture.

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5.4.3 VACCINE APPROACHES

In certain embodiments, a vector of the invention comprising an expression cassette of a DNA sequence of interest is administered to a subject to induce an immune response. Specifically, the DNA sequence of interest encodes a protein (for example, a peptide or polypeptide), which induces a specific immune response upon its expression. Examples of such proteins are discussed in section 10 5.3.

For the delivery of a vector of the invention for use as a vaccine, methods may be selected from among those known in the art and/or described in section 15 5.4.6.

5.4.4 GENE THERAPY APPROACHES

In a specific embodiment, a vector of the invention comprising an expression cassette comprising DNA sequences of interest is administered to treat, or prevent various diseases. The DNA sequence of interest may encode a protein 20 and/or a biologically active RNA molecule. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible DNA sequence. In this embodiment of the invention, the DNA sequences produce their encoded protein or RNA molecule that mediates a therapeutic effect.

25 Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the method of gene therapy, see, Goldspiel et al., Clinical Pharmacy 12 (1993) 488-505; Wu and Wu, Biotherapy 3 (1991) 87-95; Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32 (1993) 573-596; Mulligan, Science 30 260 (1993) 926-932; Morgan and Anderson, Ann. Rev. Biochem. 62 (1993) 191-217; May, TIBTECH 1, I(5) (1993) 155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et

al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

The following animal regulatory regions, which exhibit tissue specificity and have been utilized in transgenic animals, can be used for expression of the DNA sequence of interest in a particular tissue type: elastase I gene control region which is active in pancreatic acinar cells [Swift et al., Cell 38 (1984) 639-646; Ornitz et al., Cold Spring Harbor Symp. Quant. Biol. 50 (1986) 399-409; MacDonald, Hepatology 7 (1987) 425-515]; insulin gene control region which is active in pancreatic beta cells [Hanahan, Nature 315 (1985) 115-122], immunoglobulin gene control region which is active in lymphoid cells [Grosschedl et al., Cell 38 (1984) 647-658; Adames et al., Nature 318 (1985) 533-538; Alexander et al., Mol. Cell. Biol. 7 (1987) 1436-1444], mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells [Leder et al., Cell 45 (1986) 485-495], albumin gene control region which is active in the liver [Pinkert et al., Genes and Devel. 1 (1987) 268-276], alpha-fetoprotein gene control region which is active in the liver [Krumlauf et al., Mol. Cell. Biol. 5 (1985) 1639-1648; Hammer et al., Science 235 (1987) 53-58; alpha 1-antitrypsin gene control region which is active in the liver [Kelsey et al., Genes and Devel. 1 (1987) 161-171], beta-globin gene control region which is active in myeloid cells [Mogram et al., Nature 315 (1985) 338-340; Kollias et al., Cell 46 (1986) 89-94]; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain [Readhead et al., Cell 48 (1987) 703-712]; myosin light chain-2 gene control region which is active in skeletal muscle [Sani, Nature 314 (1985) 283-286], and gonadotropic releasing hormone gene control region which is active in the hypothalamus [Mason et al., Science 234 (1986) 1372-1378].

Methods of delivery for gene therapy approaches are well known in the art and/or described in section 5.4.6.

30 5.4.5 INHIBITORY ANTISENSE AND RIBOZYME

In certain embodiments of the invention a vector of the invention contains a DNA sequence of interest that encodes an antisense or ribozyme RNA mole-

cule. Techniques for the production and use of such molecules are well known to those of skill in the art.

Antisense RNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. Antisense approaches involve the design of oligonucleotides that are complementary to a target gene mRNA. The antisense oligonucleotides will bind to the complementary target gene mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required.

A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with at least the non-polyA portion of an RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides. In other embodiments of the invention, the antisense nucleic acids are at least 100, at least 250, at least 500, and at least 1000 nucleotides in length.

Regardless of the choice of target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally, it is envisioned that results obtained using the antisense DNA sequence

are compared with those obtained using a control DNA sequence. It is preferred that the control DNA sequence is of approximately the same length as the test oligonucleotide and that the DNA sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization 5 to the target sequence.

While antisense DNA sequences complementary to the target gene coding region sequence could be used, those complementary to the transcribed, untranslated region are most preferred.

For expression of the biologically active RNA, e.g., an antisense RNA 10 molecule, from the vector of the invention the DNA sequence encoding the biologically active RNA molecule is operatively linked to a strong pol III or pol II promoter. The use of such a construct to transfet target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous target gene transcripts and thereby prevent translation of the target gene mRNA. For example, a vector of 15 the invention can be introduced, e.g., such that it is taken up by a cell and directs the transcription of an antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to 20 act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region [Benoist and Chambon, Nature 290 (1981) 304-310], the promoter contained in the 3 long terminal repeat of Rous sarcoma virus [Yamamoto, et al., Cell 22 (1980) 787-797], the herpes thymidine kinase promoter [Wagner, et al., 25 Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 1441-1445], the regulatory sequences of the metallothionein gene [Brinster, et al., 1982, Nature 296 (1982) 39-42], etc.

In certain embodiments of the invention, a vector of the invention contains 30 a DNA sequence, which encodes a ribozyme. Ribozyme molecules designed to catalytically cleave target gene mRNA transcripts can also be used to prevent translation of a target gene mRNA and, therefore, expression of a target gene product [see, e.g., PCT International Publication WO90/11364, published October 4, 1990; Sarver, et al., Science 247 (1990) 1222-1225].

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. [For a review, see Rossi, Current Biology 4 (1994) 469-471]. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, e.g., U.S. Patent No. 5,093,246, which is incorporated herein by reference in its entirety.

While ribozymes that cleave mRNA at site-specific recognition sequences can be used to destroy target gene mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Myers, 1995, Molecular Biology and Biotechnology: A Comprehensive Desk Reference, VCH Publishers, New York, (see especially Figure 4, page 833) and in Haseloff & Gerlach, Nature, 334 (1988) 585-591, which is incorporated herein by reference in its entirety.

Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the target gene mRNA, i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA) and that has been extensively described by Thomas Cech and collaborators [Zaug, et al., Science, 224 (1984) 574-578; Zaug and Cech, Science, 231 (1986) 470-475; Zaug, et al., Nature, 324 (1986) 429-433; published International patent application No. WO 88/04300 by University Patents Inc.; Been & Cech, Cell, 47 (1986) 207-216]. The Cech-type ribozymes have an eight base pair active site, which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The

invention encompasses those Cech-type ribozymes, which target eight base-pair active site sequences that are present in the target gene.

Expression of a ribozyme can be under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities 5 of the ribozyme to destroy endogenous target gene messages and inhibit translation. Because ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

In instances wherein the antisense and/or ribozyme molecules described herein are utilized to inhibit mutant gene expression, it is possible that the technique 10 may so efficiently reduce or inhibit the translation of mRNA produced by normal target gene alleles that the possibility may arise wherein the concentration of normal target gene product present may be lower than is necessary for a normal phenotype. In such cases, to ensure that substantially normal levels of target gene activity are maintained, therefore, nucleic acid molecules that encode and 15 express target gene polypeptides exhibiting normal target gene activity may be introduced into cells via gene therapy methods such as those described, below, in Section 5.4.4 that do not contain sequences susceptible to whatever antisense, ribozyme, or triple helix treatments are being utilized. Alternatively, in instances whereby the target gene encodes an extracellular protein, it may be preferable to 20 co-administer normal target gene protein in order to maintain the requisite level of target gene activity.

Methods of administering the ribozyme and antisense RNA molecules are well known in the art and/or described in section 5.4.6.

25 5.4.6 PHARMACEUTICAL FORMULATIONS AND MODES OF
ADMINISTRATION

In a preferred aspect, a pharmaceutical of the invention comprises a substantially purified vector of the invention (e.g., substantially free from substances 30 that limit its effect or produce undesired side-effects). The subject to whom the pharmaceutical is administered in the methods of the invention is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably a human.

In certain embodiments, the vector of the invention is directly administered *in vivo*, where the DNA sequence of interest is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art. The vectors of the invention can be administered so that the nucleic acid sequences become intracellular. The vectors of the invention can be administered by direct injection of naked DNA; use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont); coating with lipids or cell-surface receptors or transfecting agents; encapsulation in microparticles or microcapsules; administration in linkage to a peptide which is known to enter the nucleus; administration in linkage to a ligand subject to receptor-mediated endocytosis [see, e.g., Wu and Wu, *J. Biol. Chem.* 262 (1987) 4429-4432] (which can be used to target cell types specifically expressing the receptors); etc. In a specific embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome [see Langer, *Science* 249 (1990) 1527-1533; Treat *et al.*, 1989, in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365; Lopez-Berestein, *ibid.*, pp. 317-327].

In certain embodiments, the vector of the invention is coated with lipids or cell-surface receptors or transfecting agents, or linked to a homeobox-like peptide which is known to enter the nucleus [see e.g., Joliot *et al.*, *Proc. Natl. Acad. Sci. USA* 88 (1991) 1864-1868], etc.

In certain other embodiments, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation.

In yet other embodiments, the vector of the invention can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06 180; WO 92/22635; W092/20316; W093/14188, and WO 93/20221).

Methods for use with the invention include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. Methods for use with the invention further include administration by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal

and intestinal mucosa, etc.). In a specific embodiment, it may be desirable to administer a vector of the invention by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including a membrane, such as a sialastic membrane, or a fiber. Care must be taken to use materials to which the vector does not absorb. Administration can be systemic or local.

In certain embodiments, a vector of the invention is administered together with other biologically active agents such as chemotherapeutic agents or agents that augment the immune system.

In yet another embodiment, methods for use with the invention include delivery via a controlled release system. In one embodiment, a pump may be used [see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14 (1989) 201; Buchwald *et al.*, Surgery 88 (1980) 507; Saudek *et al.*, N. Engl. J. Med. 321 (1989) 574]. In another embodiment, polymeric materials can be used [see Medical Applications of Controlled Release, 1974, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida; Controlled Drug Bioavailability, Drug Product Design and Performance, 1984, Smolen and Ball (eds.), Wiley, New York; Ranger and Peppas, Macromol. Sci. Rev. Macromol. Chem. 23 (1983) 61; see also Levy *et al.*, Science 228 (1985) 190; During *et al.*, Ann. Neurol. 25 (1989) 351; Howard *et al.*, J. Neurosurg. 71 (1989) 105].

Other controlled release systems are discussed in the review by Langer, Science 249 (1990) 1527-1533.

Pharmaceutical compositions of the invention comprise a therapeutically effective amount of a vector of the invention, and a suitable pharmaceutical vehicle. In a specific embodiment, the term "suitable pharmaceutical vehicle" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "vehicle" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such suitable pharmaceutical vehicles can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier

when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel,
5 sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations
10 and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the nucleic acid or protein of the invention, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.
15
20 In a specific embodiment, the pharmaceutical of the invention is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the pharmaceutical of the invention may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water
25 free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the pharmaceutical of the invention is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the pharmaceutical of the invention is administered by injection, an ampoule of sterile water
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for injection or saline can be provided so that the ingredients may be mixed prior to administration.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

5 For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol 10 the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

15 The amount of a vector of the invention, which will be effective in the treatment or prevention of the indicated disease, can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the stage of indicated disease, and should be decided according to the judgment of the practitioner 20 and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

25 The present invention may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the invention. The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

6. EXAMPLES

6.1. EXAMPLE 1

30 Cloning and analysis of the expression properties of the vectors super6 and super6wt

The vector plasmids super6 (Figure 1) and super6wt were prepared from previous generation based gene vaccination vectors VI (Figure 2) and VIwt, respectively. Vectors VI and VIwt are principally synthetic bacterial plasmids that contain a transposon Tn903 derived kanamycin resistance marker gene [Oka, A., et al., J Mol Biol 147 (1981) 217-226] and a modified form of pMB1 replicon [Yanisch-Perron, C., et al., Gene 33 (1985) 103-119] needed for the propagation in Escherichia coli cells. Vectors VI and VIwt also contain a Cytomegalovirus Immediately Early Promoter combined with a HSV1 TK leader sequence and rabbit β-globin gene sequences, which both are derived from plasmid pCG [Tanaka, M., et al., 60 (1990) Cell 375-386]. The latter elements are needed for expressing from the nef coding sequence derived from a HAN2 isolate of the HIV-1 strain [Sauermann, U., et al., AIDS Research. Hum. Retrov. 6 (1990) 813-823]. The expression vectors for the Nef carry clustered ten high affinity E2 binding sites (derived from plasmid pUC1910BS, unpublished) just upstream of the CMV promoter.

The parent vector VI contains a modified E2 coding sequence: the hinge region of E2 (amino acids 192-311) is replaced with four glycine-alanine repeats from EBNA1 protein of Epstein-Barr Virus [Baer, R. J., et al., Nature 310 (1984) 207-211]. The protein encoded by this sequence was named as E2d192-311+4G. The parent vector VIwt contains an expression cassette for wild type E2 protein of the bovine papilloma virus type 1 with point mutations introduced for the elimination E3 and E4 open reading frame (ORF) coding capacity by two stop codons into both these ORFs. In the vectors the E2 coding sequences are cloned between a Rous sarcoma virus proviral 5' LTR [Long, E. O., et al., Hum. Immunol. 31 (1991) 229-235] and bovine growth hormone polyadenylation region [Chesnut, J. D., et al., J Immunol Methods 193 (1996) 17-27].

Plasmid vectors super6 and super6wt were constructed by deleting from the respective parent vectors VI and VIwt all beta-globin sequences downstream of the nef gene except the second intron of the rabbit beta-globin gene. The beta-globin sequences (especially the fragment of the exon) show some homology with sequences in the human beta-globin gene, whereas the intron lacks any significant homology to human genomic sequences. The intron was amplified by

PCR from the plasmid pCG [Tanaka, M. et al., Cell 60 (1990) 375-386] using oligonucleotides with some mismatches for modifying the sequences of splicing donor and acceptor sites of the intron to the perfect match to consensus motifs. The Herpes Simplex Virus type 1 thymidine kinase gene polyadenylation region from 5 pHock [Chesnut, J. D., et al., J Immunol Methods 193 (1996) 17-27] was then cloned just next to the 3'-end of the intron, because in parent plasmids the rabbit β-globin polyadenylation signal were used.

The expression properties of the Nef and E2 proteins expressed by the plasmid vectors super6 and super6wt were analyzed and compared with the expression properties of the Nef and E2 proteins expressed by VI and VIwt by Western blotting [Towbin et al., Proc Natl Acad Sci USA 76 (1979) 4350-4354] with monoclonal antibodies against Nef and E2. First, Jurkat cells (a human T-cell lymphoblast cell line) were transfected by electroporation [Ustav et al. EMBO J 2 (1991) 449-457] with 1 µg of super6, super6WT or equimolar amounts of the 10 plasmids VI, VIwt. As a control an equimolar amount of vector II (Figure 3), which contains an identical Nef cassette but no E2 coding sequence, was used. Carrier 15 DNA was used as a negative control. Briefly, the plasmid and carrier DNA were mixed with the cell suspension in a 0.4 cm electroporation cuvette (BioRad Laboratories, Hercules, USA) followed an electric pulse (200V; 1mF) using Gene Pulser II TM with capacitance extender (BioRad Laboratories, Hercules, USA).

Forty-nine hours post-transfection the cells were lysed by treating with a sample buffer containing 50mM Tris-HCl pH 6.8; 2% SDS, 0.1% bromophenol blue, 100mM dithiothreitol, and 10% (v/v) glycerol. The lysates were run on a 10% or 12.5% SDS-polyacrylamide gel and subsequently transferred onto a 0.45 20 µm PVDF nitrocellulose membrane (Millipore). The membrane was first blocked overnight with a blocking solution containing 5% dry milk (fat-free), 0.1% Tween 20 in 50 mM Tris-HCl pH 7.5; 150mM NaCl and thereafter incubated for 1h with 25 diluted monoclonal anti-Nef antisera (1:100) or anti-E2-antisera (1:1000) in the blocking solution. After each incubation step, unbound proteins were removed by 30 washing strips three times with TBS - 0.1 % Tween-20. The binding of primary immunoglobulins was detected by incubating the strips with horse raddish peroxidase conjugate anti-mouse IgG (Labas, Estonia) followed by visualization using a

chemoluminescence detection system (Amersham Pharmacia Biotech, United Kingdom).

The results are shown in Figure 4. The expression of the Nef protein is shown on panel A and the expression of E2 protein on panel B. The arrows indicate the right molecular sizes of the Nef and E2 proteins. The expression level of the E2d192-311+4GA is very low and for this reason cannot be seen on the blot presented in Figure 4.

The amounts of Nef expressed from the plasmids super6, super6wt, VI and VIwt (lanes 1-4 in Figure 4A) are quite similar (Figure 4, panel A, lanes 1 to 10 4). Much less protein is produced from plasmid II (lane 5). The expression levels 15 of the Nef protein are higher from vectors containing wtE2 (cf. lane 1 compared with lane 2 and lane 3 compared with lane 4). This is in accordance with the expression levels of E2 and E2d192-311+4GA proteins from these plasmids (Figure 4, panel B).

15

6.2. EXAMPLE 2

Cloning and analysis of the expression properties of plasmids in series product1 and NNV

To increase the copy number of the vectors super 6 and super6wt in Escherichia coli further modifications were made in these vectors. The Tn903 kanamycin resistance gene, pMB1 replicon and ten E2 binding sites were removed by HindIII/NheI digestion followed by replacing with the Hind III/NheI fragment from retroviral vector pBabe Neo [Morgenstern, J.P. and Land, H., Nucleic Acids Research 18 (1990) 3587 - 3596]. This fragment contains a modified pMB1 20 replicon and the Tn5 kanamycin resistance gene that allow relaxed high copy-number replication of the plasmids in bacteria. The new plasmids were named as the product1 (Figure 5), and product1wt respectively. An unsuccessful attempt to 25 reinsert the ten E2 binding sites back into the blunted NheI site upstream of the CMV promoter of the product1 resulted in vector New Vector NNV, respectively, 30 with only two binding sites integrated in the plasmid.

Additional ten E2 binding sites were inserted from plasmid pUC1910BS into the New Vector in just downstream the E2 expression cassette. These new

vectors were named NNV-1 and NNV-2 (Figure 6A). For replacing the E2d192-311+4GA with wt E2 (with deleted E3 and E4 ORFs), the E2d192-311+4GA coding sequence containing Bsp120I fragment was replaced with wtE2 containing an analogous Bsp120I fragment from the super6wt. Generated plasmids were 5 named NNV-1wt and NNV-2wt (Figure 6B), respectively. The numbers 1 or 2 in vectors of the NNV series mark the orientation of the 10 E2 binding sites region relative to the E2 expression cassette.

The expression properties of the Nef protein from the NNV plasmids, i.e. NNV-1, NNV-2, NNVwt and NNV-2wt, after the transfection of Jurkat cells by 10 electroporation at a concentration of 1 lg of the plasmid were analyzed and compared with the expression properties of the Nef proteins from super6 and super6wt by Western blotting essentially as described in Example 1. The amounts of super6 and super6wt used for the transfection were 0,95 and 1 lg, respectively. The results are shown in Figure 7.

15 NNV-1 and NNV-2 vectors have expression potential similar to plasmid super6 as evident from the comparison of lanes 1 and 2 on figure 8 with lane 5. The same applies to vectors NNV-1wt, NNV-2wt and super6wt (compare lanes 3 and 4 with lane 6 on figure 7). In accordance with the previous results the plasmids expressing wt E2 produce more Nef protein than E2d192-311+4GA vectors 20 do (compare lane 1 with lane 3 and lane 2 with lane 4 in figure 7). In view of this and since the Nef expression from NNV-2wt was slightly higher than that from NNV-1wt, vector NNV-2wt was selected for further tests.

6.3 EXAMPLE 3

25 Analysis of the expression properties of NNV-2wt

To analyse the expression properties of NNV-2wt, four different cell lines, i.e. the Jurkat (human T-cell lymphoblasts), P815 (mouse mastocytoma cells), CHO (Chinese Hamster Ovary cells) lines and RD (human embryo rhabdomyo-sarcoma cells), were transfected by electroporation and analyzed for their expression of Nef of and E2. To reveal the transcription activation and maintenance properties mediated by E2 protein and E2 oligomerized binding sites product1wt, 30 which lacks the E2 binding sites (Figure 5), was used as a control. An additional

control plasmid was plasmid NNV-2wtFS, which differs from NNV-2wt by containing a frameshift introduced into E2 coding sequence, whereby it does not express functional E2 protein.

Each cell line was transfected with different amounts of the vector DNA by 5 electroporation essentially as described in Example 1. Time-points were taken approximately two and five days after transfection. The results of analyses are presented in figures 8 to 10.

The Jurkat cells were transfected with 0.5 µg or 2 µg of the NNV-2wt (lanes 1,2, 8, and 9 in Figure 8) and equal amounts of the plasmids NNV-2wtFS 10 (lanes 3, 4, 10, and 11 in Figure 8) and product1wt (lanes 5, 6, 12, and 13 in Figure 8) or carrier only (lanes 7 and 14 in Figure 8). Time-points were taken 44 hours (lanes 1-7) and 114 hours (lanes 8-14) after transfection: The expression of the Nef and E2 proteins was analyzed by Western blotting essentially as described in Example 1.

15 The P815 cells were transfected with 0.5 µg or 2 µg of the NNV-2wt (lanes 1,2, 8, and 9 in Figure 9) and equal amounts of the plasmids NNV-2wtFS (lanes 3, 4, 10, and 11 in Figure 9) and product1wt (lanes 5, 6, 12, and 13 in Figure 9) or carrier only (lanes 7 and 14 in Figure 9). Time-points were taken 45 hours (lanes 1-7) and 119 hours (lanes 8-14) after transfection: The expression of the Nef 20 proteins was analyzed by Western blotting essentially as described in Example 1. The blot with anti-E2 antibodies 119h post-transfection is not shown, because no special signal could be detected. Generally, the expression level of the Nef protein correlated with the expression level of E2 protein in these cells, which confirms the fact that the function of the E2 protein is to activate the transcription and 25 to help the plasmid to be maintained for a longer time in the proliferating cells.

The CHO cells were transfected with 0.5 µg or 2 µg of the NNV-2wt (lanes 1,2, 8, and 9 in Figure 10) and equal amounts of the plasmids NNV-2wtFS (lanes 3, 4, 10, and 11 in Figure 10) and product1wt (lanes 5, 6, 12, and 13 in Figure 10) or carrier only (lanes 7 and 14 in Figure 10). Time-points were taken 48 hours 30 (lanes 1-7) and 114 hours (lanes 8-14) after transfection. The expression of the Nef and E2 proteins was analyzed by Western blotting essentially as described in Example 1.

The RD cells were transfected with 0.5 µg or 2 µg of the NNV-2wt (lanes 1, 2, 8, and 9 in Figure 11) and equal amounts of the plasmids NNV-2wtFS (lanes 3, 4, 10, and 11 in Figure 11) and product1wt (lanes 5, 6, 12, and 13 in Figure 11) or carrier only (lanes 7 and 14 in Figure 11). Time-points were taken 39 hours 5 (lanes 1-7) and 110 hours (lanes 8-14) after transfection. The expression of the Nef protein was analyzed by Western blotting essentially as described in Example 1.

In all four cell lines the expression level of the Nef protein, taken at earlier time points (lanes 1-7 in figures 8 - 11) and at later time points (lanes 8-14 in Figures 8-11) hours, from the NNV-2wt was higher than from control vectors. The superiority of the NNV-2wt was more obvious at later time-points as evident from the comparision of lane 8 with lanes 10 and 12 in Figure 8, and also from the comparision of lane 9 with lanes 11 and 13 in figures 8, 9 and 10.

The expression pattern of RNA from these plasmids was also analyzed using the Northern analysis [Alwine, J. C, et al., Proc Natl Acad Sci U S A 74 (1977) 5350-5354] for the NNV-2wt vector. For this purpose, Jurkat and CHO cells were transfected with 2 µg of the NNV-2wt. For the transfection of P815 cells 10 µg of NNV-2wt were used. The transfections were made essentially as described in Example 1. Forty-eight hours post-transfection total RNA was extracted using 15 RNAeasy kit (Qiagen) and samples containing 21 µg (P815), 15 µg (CHO) or 10 µg (Jurkat) of the RNA were analysed by electrophoresis under the denaturing conditions (1.3% agarose gel containing 20mM MOPS pH 7.0; 2mM NaOAc; 1mM EDTA pH 8.0; 2.2M formaldehyde). The running buffer contained the same components except formaldehyde. The samples were loaded in a buffer containing 20 formamide and formaldehyde. After the electrophoresis the separated RNAs 25 were blotted onto the HybondN+ membrane (Amersham Pharmacia Biotech, United Kingdom) and hybridization with a radio-labeled nef coding sequence, E2 coding sequence or whole vector probes was carried out. The RNA from cells transfected with the carrier was used as a control. The results of the Northern blot analyses are shown in figure 12.

The results indicate that no other RNA species than complementary mRNAs for E2 and nef are expressed from the vector, since no additional signals

can be detected with the whole vector probe compared with nef and E2 specific hybridizations (compare lanes 1-12 with lanes 13-18 in Figure 12).

6.4 EXAMPLE 4

5 Analysis of the attachment of the NNV-2wt to mitotic chromosomes

The attachment of the NNV-2wt to mitotic chromosomes in CHO cells was analyzed by fluorescence in situ hybridisation (FISH) [Tucker J.D., et al., In: J.E.Celis (ed.), Cell Biology: A Laboratory Handbook, vol 2, p. 450-458. Academic Press, Inc. New York, NY. 1994.].

10 Thirty-six hours post-transfection the CHO cells by electroporation with 1 µg of NNV-2wt or with equimolar amounts of the control plasmids NNV-2wtFS and product1wt (performed essentially as described in Example 1) the cultures were treated with colchicin (Gibco) for arresting the cells in metaphase of the mitosis. Briefly, cells were exposed to colchicine added to medium at final concentration of 0.1 µg/ml for 1-4 h to block the cell cycle at mitosis. Blocked cells were harvested by a trypsin treatment and suspended in a 0.075M KCl solution, incubated at room temperature for 15 min, and fixed in ice-cold methanol-glacial acetic acid (3:1, vol/vol). The spread-out chromosomes at metaphase and nuclei at interphase for fluorescence in situ hybridization analyses were prepared by dropping the cell suspension on wet slides. Several slides from one culture were prepared.

20 Hybridization probes were generated by nick-translation, using biotin-16-dUTP as a label and plasmid Product1wt as template. A typical nick-translation reaction mixture contained a nick-translation buffer, unlabeled dNTPs, biotin-16-dUTP, and E.coli DNA polymerase.

25 Chromosome preparations were denatured at 70°C in 70% formamide (pH 7.0-7.3) for 5 min, then immediately dehydrated in a series of washes (70%, 80%, and 96% ice-cold ethanol washes for 3 min each), and air-dried. The hybridization mixture (18 µl per slide) was composed of 50% formamide in 2xSSC (1xSSC is 0.15 M NaCl plus 0.015 M sodium citrate), 10% dextran sulfate, 150 ng of biotinylated plasmid probe DNA and 10 µg of herring sperm carrier DNA. After 5 min of denaturation at 70°C, probe DNA was applied to each slide, sealed under

a coverslip, and hybridized for overnight at 37°C in a moist chamber. The slides were washed with three changes of 2xSSC, and 2xSSC containing 0.1% IGEPAL CA-630 (Sigma Chemical Co.) at 45°C. Prior to the immunofluorescence detection, slides were preincubated for 5 min in PNM a buffer [PN buffer (25.2 g Na₂HPO₄•7 H₂O, 0.83 g NaH₂PO₄ • H₂O and 0.6 ml of IGEPAL CA-630 in 1 liter of H₂O) with 5% nonfat dried milk and 0.02% sodium azide].

Subsequently, the probe was detected with fluorescein isothiocyanate (FITC)-conjugated extravidin. The signal was amplified with biotinylated antiavidin antibody and a second round of extravidin-FITC treatment. Between each of the steps, the slides were washed in PN buffer containing 0.05% IGEPAL CA-630 at room temperature for 2x5 min. Chromosomes were counterstained with propidium iodide and mounted in p-phenylenediamine antifade mounting medium.

Slides were analyzed with a Olympus VANOX-S fluorescence microscope equipped with appropriate filter set.

The results are shown in Table 1.

TABLE 1. Chromosomal attachment of the NNV-2wt.

Culture	Meta-phases with epi-somal signal on chromosomes	Analyzed metaphases	%
0.5µg NNV-2wt	11	158	7
0.5µg NNV-2wtFS	0	100	0
0.48µg product1wt	0	100	0
carrier	0	100	0

The data indicate clearly that the E2 protein and its binding sites are needed for the chromosomal attachment because only the NNV-2wt but not two other vectors have this ability.

5 **6.5 EXAMPLE 5**

Stability of NNV-2wt during propagation in bacterial cells

The stability of NNV-2wt during propagation in bacterial cells was tested. The plasmid NNV-2wt was mixed with competent Escherichia coli cells of the DH5alpha strain [prepared as described in Inoue, H., et al., Gene 96 (1990) 23-28] and incubated on ice for 30 minutes. Subsequently, the cell suspension was subjected to a heat-shock for 3 minutes at 37°C followed by a rapid cooling on ice. One milliliter of LB medium was added to the sample and the mixture was incubated for 45 minutes at 37°C with vigorous shaking. Finally, a portion of the cells was plated onto dishes containing LB medium with 50 µg/ml of kanamycin. On the next day, the cells from a single colony were transferred onto the new dishes containing the same medium. This procedure was repeated until four generations of bacteria had been grown, and the plasmid DNA from the colonies of each generation was analyzed.

One colony from each generation was used for an inoculation of 2 ml LB medium containing 50 µg/ml of kanamycin followed by an overnight incubation at 37°C with vigorous shaking. The cells were harvested and the plasmid DNA was extracted from the cell using classical lysis by boiling. [Sambrook, S., et al., Molecular Cloning A Laboratory Manual. Second ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York]. The samples were digested with restriction endonuclease XbaI (Fermentas, Lithuania) and analyzed by agarose gel electrophoresis in comparison with the original DNA used for transformation. The results are shown in Figure 13.

As can be seen in Figure 13, the vector is stable during the passage in Escherichia coli cells: no colonies with re-arrangements were observed when compared with the DNA used for transformation (lane 9).

6.6 EXAMPLE 6

Stability of NNV-2wt in eukaryotic cells

The stability of the plasmid NNV-2wt as a non-replicating episomal element was also analyzed in eukaryotic cells. For this purpose the CHO and Jurkat cells were transfected with 2 µg of NNV-2wt. Total DNAs of the cells were extracted at 24, 72 or 96 hours post-transfection. Briefly, the cells were lysed in 20mM Tris-HCl pH 8.0; 10mM EDTA pH 8.0; 100mM NaCl; 0.2% SDS; in presence of 200 µg/ml of proteinase K (Fermentas, Lithuania). Next, the samples were extracted sequentially with phenol and with chloroform and precipitated with ethanol. The nucleic acids were resuspended in 10mM Tris-HCl pH 8.0; 1mM EDTA pH 8.0; 20 µg/ml of RNase A (Fermentas, Lithuania) and incubated for 1 hour at 37°C. Finally the DNA was re-precipitated with ammonium acetate and ethanol, washed with 70% ethanol and resuspended in 10mM Tris-HCl pH 8.0; 1mM EDTA pH 8.0. The samples were digested with different restriction endonucleases: with Eco81I (Fermentas, Lithuania) that has two recognition sites on the plasmid, with HindIII (Fermentas, Lithuania) that does not cut the NNV-2wt DNA and with DpnI (New England Biolabs, USA) that digest only DNA synthesized in Escherichia coli cells. Restricted DNAs were separated on TAE agarose electrophoresis and analyzed by Southern blotting [Southern, E.M. J. Mol. Biol. 98 (1975) 503-517] with a vector specific radiolabeled probe. The results are illustrated on figure 14. As obvious from comparison of the fragment sizes of Eco81I digestion (lanes 1, 2 and 7 in figure 14) with respective marker lanes no arrangements of the vector were detected in the assay. Neither were signals observed at a position different from the marker lanes in cases of the Hind III (lanes 3, 4 and 8 in figure 14) or HindIII/DpnI (lanes 5, 6 and 9 in figure 14) digestion indicating that integration and/or replication events were not observed.

6.7 EXAMPLE 7

Analysis of replication of the NNV-2wt in the presence of human papillomaviral replication factors

It has been demonstrated previously that papillomaviral proteins are able to initiate the replication of heterologous ori-containing plasmids from many other

human and animal papillomaviruses [Chiang, C. M., et al., Proc Natl Acad Sci U S A 89 (1992) 5799-5803]. Although NNV-2wt does not contain an intact viral origin of replication, it was tested how the replication is initiated in the presence of human papillomavirus type 1 E1 and E2 proteins. CHO cells were transfected with 5 one microgram of either plasmids NNV-2wt, NNV-2wtFS or product 1 alone or with 4.5 µg of the HPV-11 E1 expression vector pMT/E1 HPV11 or with same amount of pMT/E1 HPV11 and 4.5 µg HPV-11 E2 protein expression vector pMT/E2 HPV 11 as indicated on the top of the figure 15. Transfections were done essentially as described in Example 1. E1 and E2 expression vectors are described previously (Chiang, C. M. et al., supra). An equimolar amount of HPV-11 10 replication origin containing plasmid HPV11ORI was transfected with the same expression vectors as a positive control.

Low-molecular weight DNA was extracted by modified Hirt lysis [Ustav, et al., EMBO J 2 (1991) 449-457] at 67 hours post-transfection. Briefly, the cells 15 washed with PBS were lysed on ice at 5 minutes by adding alkaline lysis solutions I (50mM glucose; 25mM Tris-HCl, pH 8.0; 10mM EDTA, pH 8.0) and II (0.2M NaOH; 1% SDS) in a ratio of 1:2 onto the dishes. The lysates were neutralized by 0.5 vol solution III (a mixture of potassium acetate and acetic acid, 3M with respect to potassium and 5M with respect to acetate). After centrifugation the supernatant was precipitated with isopropanol, resuspended and incubated at 55°C 20 in 20mM Tris-HCl pH 8.0; 10mM EDTA pH 8.0; 100mM NaCl; 0.2% SDS; in presence of 200 µg/ml of proteinase K (Fermentas, Lithuania). Next, the samples were extracted sequentially with phenol and with chloroform followed by precipitation with ethanol. The nucleic acids were resuspended in 10mM Tris-HCl pH 8.0; 25 1mM EDTA pH 8.0; 20 µg/ml RNase A (Fermentas, Lithuania) and incubated for 30 min at 65°C. The samples were digested with linearizing endonuclease (NdeI; Fermentas, Lithuania) in case of the vectors or HindIII (Fermentas, Lithuania) in case of the HPV11ORI and DpnI (New England Biolabs, USA) (breaks non-replicated DNA), followed by Southern blotting performed essentially as described 30 earlier using a vector specific radiolabeled probe. For positive control of hybridization appropriate markers of the linearized vectors and HPV11ORI were used (la-

(lanes marked as M on figure 15). As seen from the results set forth in figure 15, no replication signal was detected in case of any vector plasmids.

6.8 EXAMPLE 8

5 **Analysis of the E2 and its binding sites dependent segregation function of the vectors in dividing cells**

As has been described previously, bovine papillomavirus type 1 E2 protein in trans and its multiple binding sites in cis are both necessary and sufficient for the chromatin attachment of the episomal genetic elements. The phenomenon is 10 suggested to provide a mechanism for partitioning viral genome during viral infection in the dividing cells [Ilves, I., et al., J Virol. 73 (1999) 4404-4412]. Because both functional elements are also included into our vector system, the aim of this study was analyze the importance of the E2 protein and oligomerized binding sites for maintenance of the transcriptionally active vector element in population of 15 dividing cells.

For this purpose the Nef coding sequence of the vectors NNV-2wt and super6wt was replaced with coding sequence of the destabilized form of green fluorescent protein (d1EGFP) derived from vector pd1EGFP-N1 (Clontech Laboratories). Because the half-life of this protein is as short as 1 hour, it does not accumulate in the cells and the d1EGFP expression detected by flow cytometer correlates with the presence of transcriptionally active vector in these cells. 20

From NNV-2wt the nef coding sequence was removed and SmaI-NotI fragment from the pd1EGFP-N1 was inserted instead of it. New vector was named as 2wtd1EGFP (Figure 16). Similar replacement was made in case of super6wt for generation gf10bse2 (Figure 17), respectively. The recognition sequence for restriction endonuclease Spel was introduced into the EcoRI site in the super6wt just upstream the ten E2BS. The vector gf10bse2 is derived from this plasmid by replacing the Nef coding sequence containing NdeI-Bst1107I fragment with d1EGFP coding sequence containing fragment from 2wtd1EGFP, cut 25 out with same enzymes.

Negative control plasmids lacking either functional E2 coding sequence or its binding sites were also made: The frameshift was introduced into the E2 cod-

ing sequence in context of the 2wd1EGFP by replacing E2 coding sequence containing Bsp120I-Bsp120I with similar fragment from plasmid NNV-2wtFS. The resulting vector was named as 2wd1EGFPFS (Figure 18). For the construction the control plasmid NNVd1EGFP (Figure 19) the whole E2 expression cartridge (as well bacterial replicon) from the 2wd1EGFP was removed by Bst1107 and NheI digestion. The replicon was reconstituted from plasmid product1 as HindIII (filled in)-NheI fragment.

Jurkat cells were transfected by electroporation with 1 μ g of the vector 2wd1EGFP or with equimolar amounts of the plasmids 2wd1EGFPFS, 10 NNVd1EGFP, gf10bse2 or with carrier DNA only as described in Example 1. At different time-points post-transfection the equal aliquots of the cell suspension were collected for analysis and the samples were diluted thereafter with the fresh medium. At every time-point total number of the cells as well the number of the d1EGFP expressing cells were counted by flow cytometer (Becton-Dickinson 15 FACSCalibur System). With these data, the percentages of d1EGFP expressing cells, alterations of total numbers of cells and numbers of d1EGFP expressing cells in samples were calculated using the carrier-only transfected cells as a negative control for background fluorescence. The calculations of cell numbers were done in consideration of the dilutions made. Finally, the error values were calculated based on technical data of the cytometer about fluctuations of speed of the 20 flow.

Two independent experiments were done. First, the maintenance of d1EGFP expressed from the plasmids 2wd1EGFP, 2wd1EGFPFS and NNVd1EGFP were analyzed during the eight days post-transfection. In the second experiment the maintenance of d1EGFP expressed from the plasmids 2wd1EGFP, 2wd1EGFPFS and gf10bse2 were analyzed during the thirteen days post-transfection.

As is obvious from Figures 20 and 21, there was no difference of the growth speed of the cells transfected with any vector or carrier only. It means 30 that differences in the d1EGFP expression maintenance are not caused by influences of transfected vectors themselves on the dividing of the cells. Also, during the assay the logarithmic growth of the cells were detected, except the period un-

til second time-point in the experiment represented in Figure 20. This lag period of the growth is probably caused by the electroporation shock of the cells, because the first time-point was taken already 19 hours after the transfection.

As illustrated in Figures 22 and 23, the percentages of green fluorescent protein expressing cells decrease in all populations transfected with either plasmid, because the vectors do not replicate in the cells. However, as is seen on the charts, the fraction of positive cells declines more rapidly in cases of control vectors, if compared with the 2wtD1EGFP or gf10bse2. If compared with each other, the gf10bse2 have clear benefit to 2wtD1EGFP (Figure 23.). There is also a notable difference of maintenance between control plasmids 2wtFSd1EGFP and NNVd1EGFP (Figure 22).

These differences between the vectors become much more obvious, if the data are represented as alterations of the numbers of the d1EGFP expressing cells in the populations (Figures 24 and 25). The numbers of the positive cells in cases of the control plasmids are not notably changed during the assay. In contrast, in case of the 2wtD1EGFP the number of d1EGFP expressing cells increases during the first week after the transfection becoming approximately five to ten times higher than in control samples (Figure 24). After this time-point the numbers start to decrease (Figure 25). The difference of maintenance is strongest in the case of the gf10bse2 vector. The number of positive cells increases continuously during the analyses period. After two weeks it is 6 times higher than in the sample transfected with 2wtD1EGFP and 45 times higher than in the population transfected with frameshift mutant (Figure 25).

The data demonstrate clearly that the vector system of the present invention has active mechanism of segregation based on a nuclear-anchoring protein, i.e. bovine papillomavirus type 1 E2 protein and its binding sites that promotes its maintenance in a population of proliferating cells as a transcriptionally active element.

6.9 EXAMPLE 9:

CLONING OF THE AIRE GENE INTO SUPER6WT AND EXPRESSION IN AN EPITHELIAL CELL LINE

The AIRE gene coding for the AIRE protein (AIRE = autoimmune regulator) is mutated in an autosomally hereditary syndrome APECED (Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy). AIRE is expressed in rare epithelial cells in the medulla of thymus and in the dendritic cells in peripheral blood and in peripheral lymphoid organs. APECED could thus be treated by transferring the non-mutated AIRE gene ex vivo to peripheral blood dendritic cells, followed by the introduction of the corrected dendritic cells back to the patient. To test this possibility human AIRE gene and the homologous murine AIRE gene were transferred to COS-1 cells.

For cloning of the AIRE gene into Super6wt a maxi-preparation of the vector was prepared. First a transfection with Super6wt was done to TOP10 -cells (chemically competent Escherichia coli by Invitrogen) according to manufacturer's protocol. Briefly, the cells were incubated on ice for 30 minutes, after which a heat shock was performed in a water bath at +42°C for 30 seconds. The cells were then transferred directly on ice for 2 minutes and grown in 250 µl of SOC-medium (2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose) at +37°C with shaking for 1 hour.

Plating was done on LB-plates using kanamycin (50 µg/ml) for selection. For maxiprep, colonies were transferred to 150 ml of LB-solution containing kanamycin (50 µg/ml) and grown overnight at +37°C with shaking. Preparation of maxiprep was done using Qiagen's Plasmid Maxi Kit according to manufacturer's protocol.

A digestion with BamHI and Sall restriction enzymes was used to check the vector. The reaction mixture contained 500 ng of Super6w, 5 U of BamHI, 5 U of Sall, 2 µl of 2XTANGO buffer (both the restriction enzymes and buffer from Fermentas) and sterile water in total volume of 10 µl. The digestion was carried out at +37°C for one hour.

The digested vector was checked with 1% agarose gel containing ethidium bromide 1 µg/ml in 1X TAE-buffer.

For cloning of the PCR amplified AIRE gene and Aire fragments into the Super6wt, 4 µg of Super6wt was digested with 10 U NotI restriction enzyme (MBI Fermentas, in 2 µl enzyme buffer and sterile water added to a final volume of 20

μl. The digestion was carried at +37°C for 1.5 hours, after which 1U of ZIP-enzyme (alkaline phosphatase) was added to the reaction mixture and incubated further for 30 minutes. The ZIP-enzyme treatment was done to facilitate the insertion of the AIRE gene into the vector by preventing the self-ligation of the vector back to a circular mode. After the digestion the vector was purified using GFXTM PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech) and dissolved in to a concentration of 0.2 micrograms/microliter.

Human and mouse AIRE-gene PCR-products were also digested with NotI restriction enzyme. To the digestion, 26 μl of PCR product, 3 μl of an appropriate 10 enzyme buffer and 10U of NotI restriction enzyme (the buffer and enzyme from MBI Fermentas) was used. The digestion was carried out at +37°C for 2 hours, after which digested PCR-products were purified and dissolved in sterile water to a volume of 10 μl.

The PCR amplified and digested human and mouse AIRE genes were ligated to Super6wt by a T4 DNA ligase (MBI Fermentas). The digested insert DNA was taken (a total volume of 10 μl), 1.5 μl of ligase buffer (MBI Fermentas), 5U of T4 DNA ligase and sterile water was added to a final concentration of 15 μl. The ligation was carried out at +17°C overnight.

After the ligation 10 μl of ligation reaction mixture was taken for transfection into TOP10 cells according to manufacturer's protocol. The cells were incubated on ice for 30 minutes, after which a heat shock was performed in a water bath at +42°C for 30 seconds. The cells were then transferred directly on ice for 2 minutes and grown in 250 μl of SOC-medium (2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose) at +37°C with shaking for 1 hour.

The transfected bacterial cells were plated onto LB-kanamycin plates and colonies were picked on the following day to 2 ml of LB-medium (1% tryptone, 0.5% yeast extract, 170 mM NaCl) with kanamycin and grown overnight at +37°C.

Miniprep DNA preparations from selected colonies were purified using 30 Qiagen's Plasmid Midi Kit and dissolved to a volume of 50 μl of sterile water. The presence and size of the insert was checked with NotI and BamHI digestion. 10 μl of miniprep DNA was taken for digestion, 5U of NotI and 5U of BamHI enzymes,

2 ml of R+ enzyme buffer and sterile water was added to a final volume of 20 µl. The digestion was carried out at +37°C for 1 hour.

The orientation of the insert was analysed with BamHI restriction enzyme. Ten µl of minprep DNA was taken, 5 U of BamHI, 2 µl of BamHI buffer (MBI Fer-
5 mentas) and sterile water was added to a final volume of 20 µl. The digestion was carried out for 1 hour at +37°C and the products were checked on a 1% agarose gel with EtBr in 1XTAE.

On the basis on these results, a plasmid containing a mouse AIRE-gene and a plasmid containing a human AIRE-gene were picked and maxipreps were
10 prepared. Briefly, 0.5 ml of E. coli cell suspension containing the plasmid of interest or a miniprep culture was added to a 150 ml LB-medium containing kanamycin (50 µg/ml) and grown overnight at +37°C. Maxiprep DNAs were prepared using Qiagen's Plasmid Maxi Kit.

The plasmid containing the mouse AIRE-gene was designated as
15 pS6wtmAIRE and plasmid containing the human AIRE-gene as pS6wthAIRE.

The generated vectors were sequenced for approximately 500 bp from both ends to verify the orientation and correctness of the insert. The sequencing was performed using the dideoxy method with PE Biosystem's Big Dye Terminator RR-mix, which contains the four different terminating dideoxynucleotide
20 triphosphates labeled with different fluorescent labels.

Plasmids containing the AIRE gene and AIRE gene fragments were inserted into selected cell lines to check the expression of the protein with Western blot after the transfection.

Cos-1 cells were harvested with trypsin-EDTA (Bio Whittaker Europe)
25 solution and suspended 10x10⁶ cells/ml into Dulbecco's MEM (Life Technologies) medium and 250 µl of cell suspension was taken for transfection. The transfection of Cos-1 cells was performed using electroporation with 2.5x10⁶ cells, 50 µg of salmon sperm DNA as a carrier and 5 µg of appropriate vector. The transfections were made with pS6wthAIRE, pS6wtmAIRE, Super6wt, pCAIRE, psiAIRE
30 and pCAIRE S1-4. pCAIRE and psiAIRE are positive human AIRE controls, pCAIRE S1-4 is a positive mouse AIRE control and Super6wt is a negative control.

The electroporation was done using Biorad's Gene Pulser with capacitance 960 μ F_d, 240 V and 1 pulse. After the pulse the cells were kept at room temperature for 10 minutes and 400 μ l of medium was added. The cells were transferred to 5 ml of medium and centrifuged for 5 minutes with 1000 rpm. Cells
5 were plated and grown for 3 days at +37°C, 5% CO₂.

The cells were harvested with trypsin-EDTA and centrifuged. Then Cells were then washed once with 500 ml of 1XPBS (0.14 mM NaCl, 2.7 mM KCl, 7.9 μ M Na₂HPO₄, 1.5 μ M KH₂PO₄). 50 μ l of PBS and 100 μ l of SDS loading buffer (5% mercaptoethanol, 16 μ M Bromphenolblue, 20 μ M Xylene Cyanol, 1.6 mM Ficoll 400) was added and cells were heated at +95°C for 10 minutes.
10

For the western blot analysis SDS-PAGE was prepared with 10% separation and 5% stacking gels in a SDS running buffer (25 mM Tris, 250 mM glycin, 0.1% SDS). Cell samples and biotinylated molecular weigh marker were loaded on the gel and electrophoresis was performed with 150 V for 1h 50 minutes. The
15 transfer of proteins to a nitrocellulose membrane was performed at 100 V for 1.5 hours at room temperature with a cooler in transfer buffer.

The membrane was blocked in 5% milk in TBS (0.05 M Tris-Cl, 0.15 M NaCl, pH 7.5) for 30 minutes at room temperature. A primary antibody mixture, anti-AIRE6.1 (human) and anti-AIRE8.1 (mouse) antibodies at a dilution of 1:100
20 in 5% milk in TBS, was added onto membrane and incubated overnight at +4°C. The membrane was washed two times with 0.1% Tween in TBS for 5 minutes and once with TBS for 5 minutes. The secondary antibody, biotinylated anti-mouse IgG at a dilution of 1:500 in 5% milk in TBS was incubated for 1 hour at room temperature. The membrane was washed and horseradish peroxidase avidin D at a dilution of 1:1000 in 5% milk in TBS was added. The membrane was
25 incubated at room temperature for 1 hour and washed. A substrate for the peroxidase was prepared of 5 ml chloronaphthol, 20 ml TBS and 10 μ l hydrogen peroxide and added onto membrane. After the development of the color the membrane was washed with TBS and dried.

30 The antibody detecting with human AIRE (anti-AIRE6.1) detected the AIRE protein expression in the prepares transfected with pS6wthAIRE, pCAIRE and psiAIRE. The antibody detecting murine AIRE detected likewise the murine

AIRE in cells transfected with pS6wtmAIRE and pCAIRE S1-4. The negative control (Super6wt) showed no AIRE/aire proteins.

5 **6.10 EXAMPLE 10: DETECTION OF CELLULAR AND HUMORAL
IMMUNE RESPONSE TOWARD HIV.1 NEF IN MICE IMMUNIZED
WITH THE NNV-NEF CONSTRUCT**

DNA IMMUNIZATIONS

To further study the induction of humoral immunity by the vectors of the inventions, 5-8 weeks old both male and female BALB/c (H-2d) mice were used.
10 For the DNA immunizations, the mice were anaesthetized with 1,2 mg of pentobarbital (i.p) and DNA was inoculated on shaved abdominal skin using plasmid DNA coated gold particles. The inoculation was made with Helios Gene Gun (Bio-Rad) using the pressure of 300 psi. The gold particles were 1 µm in diameter, ~1 µg of DNA/cartridge.. The mice were immunized twice (on day 0 and day 7) with a
15 total amount of DNA of 0,4 or 8 µg/mouse. The control mice were immunized with 8 µg of the plain vector without the nef-gene, i.e.NNV-deltanef.

A blood sample was taken from the tail of the mice two weeks after the last immunization. The mice were sacrificed four weeks after the last immunization and blood samples (100 µl) were collected to Eppendorf tubes containing 10 µl of
20 0,5 M blotting (++ vs. +) and in ELISA (higher OD, more mice in higher-dose above cut-off EDTA. The absolute number of leukocytes/ml of blood was calculated from these samples for each mouse. The sera were collected for antibody assays and stored at -20°C. The spleens were removed aseptically, weighted and then homogenized to single cell suspensions for use in T, B and NK cell assays and staining.
25

Detection of the humoral immunogenicity of the vectors of the invention

For the detection of Nef-specific antibodies by Western blotting, serum samples from mice immunized with the vector constructs of the invention were diluted 1:100 to 5% milk in TBS and applied on nitrocellulose strips made with recombinant HIV-1 Nef protein. For the preparation of the nitrocellulose strips, the purified recombinant protein was boiled in a sample buffer containing 1% SDS
30

and 1% 2-mercaptoethanol, then run on a 10 or 12.5 % polyacrylamide gel and subsequently transferred onto a 0.45 µm nitrocellulose paper. The strips were first blocked with 2% BSA in 5% defatted milk-TBS and thereafter incubated with diluted sera (1:100) overnight. After incubation, unbound proteins were removed
5 by washing the strips three times with TBS - 0.05% Tween-20 and twice with water. After washings, the strips were probed with a 1:500 dilution of biotinylated anti-mouse IgG (Vector Laboratories, USA) for 2 hour. After further washings, horseradish peroxidase-avidin in a dilution of 1:1000 (Vector Laboratories, USA) was added for 1 h, the strips were washed again and the bound antibodies were
10 detected with a hydrogen peroxidase substrate, 4-chloro-1-naphtol (Sigma, USA).

The sera were also tested in ELISA to determine the exact antibody titers induced by each construct. Nef antibody ELISA was performed as previously described (Tähtinen et al., 2001). Briefly, Nunc Maxi Sorp plates were coated with 50 ng of Nef (isolate HAN), blocked with 2% BSA in phosphate buffered saline (PBS), and the sera in a dilution of 1:100 to 1:25000 were added in duplicate wells for an overnight incubation. After extensive washings, the secondary antibody, peroxidase conjugated anti-mouse IgG or IgM (DAKO), was added, and the plates were incubated for two hours and then washed. Color intensity produced from the substrate (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, ABTS, Sigma) in a phosphate-citrate buffer was measured at 405 nm using a Labsystems Multiscan Plus ELISA-plate reader. The optical density cut-off value for positive antibody reactions was determined as follows:

$$\text{cut-off} = \text{OD (x1 control mice sera)} + 3 \text{ SD.}$$

Detection of the cellular immunogenicity of the vectors of the invention

To analyze the capacity of the vectors of the invention to induce cellular immunity, T-cell and B-cell assays as well as cell surface staining were performed.

T cell proliferation assay. The spleen cells were suspended to a final concentration of 1 x 10⁶/ml RPMI-1640 (GibcoBRL) supplemented with 10% FCS (GibcoBRL), 1% penicillin-streptomycin (GibcoBRL) and 50 µM beta-mercaptoethanol (Sigma). Cells were incubated in microtitre plates at 200 µl/well with media only or with different stimuli. The final concentrations of stimuli were:

Con A 5 µg/ml, HIV-Nef-protein at a concentration of 1 and 10 µg/ml, and a negative control antigen HIV-gag at a concentration of 1 and 10 µg/ml. All reactions were made in quadruplicates. On the sixth day of the incubation 100 µl of supernatant from each well was collected and stored at -80°C for cytokine assays. Six hours before harvesting 1 µCi of 3H-thymidine (Amersham Pharmacia Biotech) was added to each well. The cells were harvested and radioactivity incorporated (cpm) was measured in a scintillation counter. The stimulation indexes (SI) were calculated as follows

$$SI = \text{mean experimental cpm} / \text{mean media cpm.}$$

10 **Lymphocyte activation.** T and B cell activation was detected by double surface staining of fresh splenocytes with anti-CD3-FITC plus anti-CD69-PE (early activation marker) and anti-CD19-FITC plus anti-CD69-PE antibodies (all from Pharmingen). Stainings were analyzed with flow cytometer (FACScan, Becton Dickinson).

15 **CTL assays.** Mouse splenocytes were co-cultured with fixed antigen presenting cells (P-815 cells infected with MVA-HIV-nef or control MVA-F6) for five days after which they were tested in a standard 4 hour 51chromium release assay [Hiserodt, J., et al., J Immunol 135 (1995) 53-59; Lagrandierie, M., et al., J Virol 71 (1997) 2303-2309] against MVA-HIV-nef infected or control target cells. In
20 CTL assays the specific lysis of 10% or more was considered positive.

25 **Cytokine assay.** IFN-gamma and IL-10 were measured from antigen-stimulated cell culture supernatants in order to analyze, whether immunized mice develop a Th1 type or Th2 response. The supernatants were collected from antigen-stimulated cells as described above. Pro-inflammatory cytokines TNF-alfa and IL-10 were measured in the sera of the immunized mice. All cytokines were measured with commercial ELISA kits (Quantikine, R&D Systems).

Spontaneous proliferation. Spontaneous splenocyte proliferation was detected by 3H-thymidine uptake of the cells cultured in the medium only for 6 days..

30 **Anti-double strand (ds) DNA antibodies.** dsDNA antibodies were measured in the sera of immunized mice, positive control mice (mrl/lpr, a generous gift from Dr. Gene Shearer, NIH, USA) and normal mice. The antibodies were as-

sayed with ELISA on poly-L-Lysine bounded lambda phage dsDNA. The results are shown in Tables 2 and 3.

Table 2 shows complete immunological results of the mice immunized with HIV-Nef plasmid DNA. Although HIV-1 Nef recombinant protein, which was used for in vitro T cell stimulation, induced some non-HIV-specific proliferation of the cells in each immunized group, there was a significant increase in the mean SI of mice immunized with 0,4 µg of the plasmid (mean SI=72,2) compared to others. Furthermore, negative control protein HIV-gag did not induce any T cell response. Only the T cells of the mice in the group that had nef-specific proliferation also produced nef-specific IFN-gamma. None of the immunized mice had cells producing IL-10, which shows that the T cell response in the immunized mice was of Th1 type and not of Th2 type. In contrast to the T cell response, mice immunized with the higher concentration of nef plasmid DNA (8 µg) had a stronger B cell response compared to mice immunized with 0,4 µg: the humoral response in mice immunized with the higher dose was detectable already three weeks after the last immunization and the response detected was stronger both in Western-). The antibodies detected belonged to IgG-class, no IgM response was detected. None of the mice developed E2 specific antibody.

The mice immunized with 0,4 µg of HIV-nef plasmid DNA had an increased number of leukocytes ($6.38 \times 10^6/\text{ml}$) in the peripheral blood compared to other groups of immunized mice and normal mice ($3.8 \times 10^6/\text{ml}$) (Table 3). The same mice had twice as much activated T cells (21%, CD3+CD69+) compared to other mice (9% and 10%). This finding is in correlation with the positive T cell response to HIV-Nef (Table 2), since the mice with a positive T cell response to Nef also had an increased number of activated T cells in their spleens. The results of Table 3 also show that none of the immunized mice developed anti-dsDNA antibodies as compared to positive control sera (OD=1,208) indicating that there is no adverse effect of the immunization.

Table 2

Mice	HIV-1 nef SI	HIV-1 gag SI	IFN-g Th1	IL-10 Th2	HIV-1 nef Ab	E2 Ab
NNV-Nef 8						
1	6	1	-	-	++	-
2	8	1	-	-	++	-
3	13	2	-	-	++	-
4	15	1	-	-	++	-
5	7	1	-	-	++	-
Mean	9.8	1.2				
NNV-NEF 0.4						
1	24	1	+	-	+	-
2	112	1	+	-	+	-
3	83	1	+	-	+	-
4	73	1	+	-	+	-
5	69	1	+	-	+	-
Mean	72.2	1				
NNV-ΔNef 8						
1	6	1	-	-	-	-
2	nt	nt	-	-	-	-
3	11	1	-	-	-	-
4	23	2	-	-	-	-
5	12	1	-	-	-	-
Mean	13	1.25				

*SI=stimulation index

nt=not tested

-, negative

+, positive

+++, strong positive

Table 3

Mice	WBC x10 ⁶ /ml	CD3 % spleen	CD3+CD69+ % spleen	CD19 % spleen	CD19+CD69+ % spleen	anti-dsDNA ab OD (1:10 dil)
NM						
1						0.355
2						0.255
3						0.231
Mean						0.280
NNV-Nef 8						
1	5	nt	nt	nt	nt	0.387
2	4.3	50	4	11	3	0.457
3	4.9	57	4	15	4	0.514
4	4.3	55	6	15	4	0.367
5	5.1	54	5	7	0	0.478
mean	4.72	54	4.75 (9%)	12	2.75	0.441
NNV-Nef 0.4						
1	3.9	nt	nt	nt	nt	0.418
2	8	41	9	18	5	0.263
3	7.5	39	8	25	9	0.375
4	5	46	9	16	6	0.285
5	7.5	43	10	13	7	0.396
mean	6.38	42.25	9 (21%)	18	6.75	0.347
NNV-Δ Nef 8						
1	4.5	61	4	9	2	0.413
2	4.6	59	4	15	1	0.353
3	3.8	50	6	17	5	0.382
4	3.1	46	7	25	8	0.448
5	3.5	nt	nt	nt	nt	0.501
mean	3.9	54	5.25 (10%)	16.5	4	0.419

Normal mouse mean WBC=3.8x10⁶/ml.

nt, not tested

a-dsDNA positive control sera OD was 1.208
(1:10 dil)

6.11. EXAMPLE 11: SAFETY AND IMMUNOGENICITY OF
A PROTOTYPE HIV VACCINE GTU-NEF IN HIV INFECTED PA-
TIENTS

5 **Production of the NNV-2-Nef vaccine (CHECK whether NNV-2
or NNVwt-2 was used)**

The investigational vaccine NNV-2-Nef was prepared according to Example 2 with the Manufacturing License No. LLDnro 756/30/2000 (issued by the Finnish National Agency for Medicines on 21.12.2000).

10 The manufacturing processes performed fulfilled the current Good Manufacturing Practices (cGMP) requirements and provided plasmid DNA preparations suitable for use in clinical phase I and II studies. The manufacturing process consisted of four steps:

- 15 a) Establishment of Master Cell Banks and Working Cell Banks
- b) Fermentation
- c) Purification
- d) Aseptic filling of the vaccine

In detail, NNV-2-Nef was produced in *E. coli* bacteria. The Master Cell Banks (MCBs) and Working Cell Banks (WCBs) containing *E. coli* DH5 alpha T1 phage resistant cell strain were established in accordance with the specific Standard Operating Procedure from pure cultured and released Research Cell Banks.

20 **a) Establishment of Master Cell Banks and Working Cell Banks**

25 The schematic procedure for establishing the cell bank system is illustrated below:

Thaw of one vial of Research Cell Bank [*E. coli* DH5 alpha T1 phage resistant cell strain (Gibco RBL) transformed with the NNV-2-Nef plasmid.

30 Inoculate of the culture on modified Luria Bertani medium plate (containing 25 µg/ml of kanamycin)

 Incubate overnight (14-16h) at 37°C

 Select of a single colony from the plate and inoculation into 50 ml of modified Luria Bertani medium (containing 25 µg/ml of kanamycin)

35 Incubate overnight (14-16h) at 37°C

 Measure optical density of the bacterial culture ($OD_{600} = 2.0 - 6.0$)

Add glycerol to bacterial culture

Divide the culture-glycerol mix to aliquots

Label and store the Master Cell Banks

Following the same diagram, the Working Cell Bank was established using one vial of the Master Cell Bank as the starting material. The routine tests performed on the MCB and WCB were: microbiological characterization, absence of contamination, assessment of the plasmid stability by replica plating and the plasmid identity (restriction enzyme digestion and sequencing).

b) **Fermentation.** In the fermentation the DH5 alpha T1 phage resistant *E. coli* strain (Gibco RBL, UK) transformed with NNV-2-Nef (WCB) was first cultured on plate. From the plate a single colony was inoculated to a 100 ml liquid pre-culture before the actual fermentation in the fermentation reactor. The fermentation was carried out in a 5 l fermentor (B. Braun Medical) on a fed-batch system basis, after which cells were harvested. The culture medium composition for one litre contained 7g of yeast extract, 8g of peptone from soy meal, 10g of NaCl, 800ml of water for injection (WFI), 1N NaOH, pH 7.0, kanamycin 50mg/ml (Sigma), silicon anti-foaming agent (Merck), 1M K₂PO₄ (BioWhittaker).

In the beginning of the fermentation run, a 1 ml sample was taken through the harvesting tube to determine the initial cell density (OD₆₀₀). The pre-culture was used to inoculate the fermentation medium. During the fermentation, fresh culture medium and 1M potassium phosphate buffer, pH 6.5 - 7.3, were fed to the reactor with the pumps. Addition of the medium allows replenishment of essential nutrients before they run out and phosphate buffer maintains the pH constant. When the fermentation process had continued for approximately 5 hours and at the end of the fermentation run (after approximately 10 hours of fermentation), samples of 1 ml were taken as above and the cell density was measured. After the fermentation, the culture medium was centrifuged (10,000 rpm, 30 minutes, +4°C) and the bacterial pellet (50-60g) was recovered.

c) **Purification.** The methodology used for the purification of DNA was based on the QIAGEN process scale technology (Qiagen Plasmid Purification Handbook 11/98). The NN2-Nef was purified using the following steps:

Resuspend the bacterial pellet in the resuspension buffer (100-150ml, RT)
Lyse with the lysis buffer (100-150ml, 5 minutes, RT)

- Neutralize with the neutralization buffers (100-150ml, +4°C)
Incubate (30minutes, +4°C)
Centrifugate (10,000 rpm, 30 minutes, +4°C)
Filtrate supernatant (0.22 micrometers)
- 5 Remove endotoxins with Endotoxin removal buffer (60-90ml)
Equilibrate Ultrapure column with Equilibration buffer (350ml, flow rate 10ml/min)
Load lysate to the column (flow rate 4-6ml/min)
Wash the column with Wash buffer (3l, overnight, flow rate 4-
- 10 6ml/min)
Elute the plasmid DNA with Elution buffer (400ml, flow rate 3.1ml/min)
Filtrate the eluate (0.22 micrometer)
Precipitate DNA with isopropanol
- 15 Centrifuge (20000g, 30 minutes, 4°C)
Purified plasmid DNA

Buffers used within the purification were as follows. The resuspension buffer contained 50mM Tris-Cl, pH 8.0, plus RNase A (50mg); the lysis buffer was 200mM NaOH; the neutralization buffer was 3M potassium acetate, pH 5.5; the endotoxin removal buffer contained 750mM NaCl, 10% Triton X-100; 50mM MOPS, pH7.0; the equilibration buffer contained 750mM NaCl, 50mM MOPS, pH 7.0; the wash buffer contained 1 M NaCl, 50mM MOPS, pH 7.0, 15% isopropanol; and elution buffer contained 1.6 M NaCl, 50mM MOPS, pH 7.0, 15% isopropanol.

25 **d) Aseptic filling**

The purified DNA representing the final bulk was dissolved in 0.9% sterile physiological saline to a final concentration of 1 mg/ml and sterile filtered (0.22 micrometer) during the same day. The purified bulk was filled 30 manually (filling volume 0.5 ml) in Schott Type 1 plus glass vials using a steam sterilized Finnpipette® and sterile endotoxin-free tips. The vials filled with the NN2-Nef vaccine were closed immediately, labelled and packed in accordance to the specific Standard Operating Procedure (SOP).

2. Administration of the test vaccine to the patients

35 Ten HIV-1 infected patients undergoing Highly Active Anti-Retroviral therapy (HAART) were immunized with the experimental DNA vac-

cine NN2-Nef, expressing the HIV-1 Nef gene (Clade B). For immunizations, two intramuscular injections in the gluteal muscle were given two weeks apart. The doses were 1 and 20 micrograms/injection. Blood samples were drawn at -4, 0, 1, 2, 4, 8 and 12 weeks. The samples were analyzed for humoral (ELISA, Western blot) and cell mediated immune response (T-cell subsets, T-cell proliferation, ELISPOT, cytokine expression, intracellular cytokines).

A clinical examination was performed to each patient participating the study. The clinical examination included a patient interview (anamnesis) and weight determination. Cardiac and pulmonar functions were checked by auscultation and percussion, the blood pressure and heart rate were recorded. Enlargement of lymph nodes, liver and thyroid gland were determined by palpation.

Laboratory tests to evaluate the safety of the vaccine were performed at each visit. These tests included:

15 Hematology: red blood cell count, haemoglobin, total and differential WBC, platelet count, prothrombin time and activated partial thromboplastin time at baseline; mean erythrocyte corpuscular volume and hemoglobin content has been calculated.

20 Immunology: nuclear and ds-DNA antibodies.

25 Serum chemistries: total bilirubin, alkaline phosphatase, SGOT/SLT or SGPT/ALT, serum creatinine, protein electrophoresis, total serum cholesterol, triglycerides, glucose (at baseline), sodium, potassium, and calcium.

30 Urine analysis: dipstick protein, glucose, ketones, occult blood, bile pigments, pH, specific gravity and microscopic examination of urinary sediment (RBC, WBC, epithelial cells, bacteria, casts), when dipstick determination showed one or more abnormal values.

Viral load: Increases of more than one log 10 should be followed by a confirmatory viral load estimate after two weeks.

35 None of the patients experienced subjective or objective adverse reactions to the vaccination. No adverse laboratory abnormalities were observed in the panel of clinical chemistry tests (see material and methods for details) performed repeatedly during the vaccination period.

The following immunological studies were performed:

Lymphocyte proliferation assay (LPA)

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by Ficoll-Hypaque density-gradient (Pharmacia) centrifugation and resuspended at 1×10^6 cells/ml in RPMI 1640 medium (Gibco) supplemented with 5% pooled, heat-inactivated AB⁺ serum (Sigma), antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin; Gibco) and L-glutamine (complete medium, CM). Quadruplicate cultures were then set up in flat-bottomed micro titer plates (1×10^5 PBMC/well) and the cells were incubated for 6 days in the presence or absence of the following stimuli: rNef (0.2, 1 and 5 µg/ml), GST (0.2, 1 and 5 µg/ml), purified protein derivative of tuberculin (PPD, 12.5 µg/ml; Statens Serum Institut), *Candida albicans* antigen (20 µg/ml; Greer Laboratories) and Phytohaemagglutinin (PHA; 5 µg/ml; Life Technologies). For the last 6 h of the incubation period 3 H-thymidine (1 µCi/well; Amersham) was added to the cultures and the cells were harvested onto glass fiber filters and incorporated radioactivity was measured in a γ-counter. Results are expressed as delta cpm (cpm in the presence of antigen/cpm without antigen) or as stimulation index (cpm in the presence of antigen/cpm without antigen).

The results are shown in Figures 26 and 27. None of the vaccinees showed significant T-cell proliferative response to the test antigen, HIV-1 Nef before the vaccination. In contrast, 2 out of 5 vaccinees in the group that had received 1 microgram dose of the test vaccine (patients 1 and 3) (Figure 26) and 2 out of 5 in the group receiving 20 micrograms of the test vaccine (patients 9 and 10) (Figure 27) showed a strong T-cell proliferative response after the first vaccination. After the second vaccination, one (patient 2) vaccinee responded in the 1-microgram group.

IFN-γ assays

The type of immune response (Th1/Th2) induced by the vaccine was evaluated by measuring interferon-gamma (IFN-γ) released in 6 days old culture supernatant after antigen (rNef, rGST, PPD) or mitogen (PHA) stimulation of PBMC. For determinations, commercial ELISA kits (R&D Quantikine) were used. The assay employ the quantitative sandwich enzyme immunoassay technique where a monoclonal antibody specific for IFN-γ has been coated onto a microplate. Standards and samples are pipetted into the wells and any IFN-γ present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific

for IFN- γ is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of the cytokine bound in the initial step. The color development is stopped and the intensity of the color is measured.

5 IFN- γ response data from patient # 1 is shown in Figure 28. As can be seen, the vaccinee responded to the rNef antigen by marked IFN- γ response correlated with the T-cell proliferation, indicating that the response seen in the vaccinee is in fact of the Th1 type.

HIV-1 infection is characterized by low or totally lacking cell-mediated
10 immune response towards all HIV proteins. The results show that it is possible to induce a robust CMI in such patients with exceptionally low doses of the DNA vaccine NN2-Nef. The doses used were minimal to what has generally been required with DNA vaccines. Thus, for instance, Merck announced recently good results with their experimental HIV vaccine but the doses required
15 were from 1000 to 5000 micrograms (IAVI report, 2002).

6.12 EXAMPLE 12: CONSTRUCTION OF THE PLASMID EXPRESSING EPSTEIN-BARR VIRUS (EBV) EBNA-1 PROTEIN AND CONTAINING 20 BINDING SITES FOR EBNA-1 (FR ELEMENT)

20 To construct a plasmid expressing Epstein-Barr virus (EBV) EBNA-1 protein and containing 20 binding sites for EBNA-1 (FR element), BPV-1 E2 binding sites were first replaced by EBV EBNA-1 binding sites (oriP without DS element). Plasmid FRE2d1EGFP (Figure 29) was constructed by isolating the *Xba*I(*Acc*I)/*Eco*32I(*Eco*RV) DNA fragment (blunt-ended with Klenow enzyme)
25 of pEBO LPP plasmid (Figure 29A) (the fragment contains 20 binding sites for EBNA-1) and inserting it by blunt end ligation into the *Sph*I/*Nhe*I site of s6E2d1EGFP (Figure 29B) (blunt-ended with Klenow enzyme). The constructed plasmid FRE2d1EGFP (Figure 29) was used as a negative control in further experiments. It contains binding sites for EBNA-1 protein instead of the
30 BPV1 E2 10 binding sites, expressing E2, but not EBNA-1.

Next, the sequence encoding BPV-1 E2 protein in FRE2d1EGFP plasmid was replaced by a sequence encoding EBV EBNA-1 protein as follows. The *Xba*I(*Acc*I)/*Eco*RI fragment of pEBO LPP plasmid was isolated and blunt-ended with Klenow enzyme and inserted into the *Xba*I/*Xba*I site of

FRE2d1EGFP plasmid (blunted, with Klenow enzyme). The vector FRE2d1EGFP was previously grown in *Escherichia coli* strain DH5 α lacking Dam methylation, because one XbaI site is sensitive for methylation. The constructed plasmid FREBNAd1EGFP (Figure 30) expresses EBNA-1 protein and contains 20 binding sites for EBNA-1.

For expression, Jurkat, human embryonic kidney cell line 293 (ATCC CRL 1573) and mouse fibroblast cell line 3T6 cells (ATCC CCL 96) were maintained in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal calf serum (FCS). Four million cells (Jurkat), 75% confluent dishes (293) or 1/4 of 75% confluent dishes (3T6) were used for each transfection, which were carried out by electroporation as follows. Cells were harvested by centrifugation (1000 rpm, 5 min, at 20 °C, Jouan CR 422), and resuspended in a complete medium containing 5mM Na-BES buffer (pH 7.5). 250 μ l cell of the cell suspension was mixed with 50 μ g of carrier DNA (salmon sperm DNA) and 1 μ g (in the case of Jurkat and 3T6) or 5 μ g (in the case of 293) of plasmid DNA and electroporated at 200 V and 1000 μ F for Jurkat cells, 170 V and 950 μ F for 293 cells and 230 V and 975 μ F for 3T6 cells. The transfected Jurkat cells were plated on 6-cm dishes with 5 ml of medium; 1/3 of transfected 293 and 3T6 cells were plated on a 6-cm dishes with 5 ml of medium and 2/3 of the cells were plated on a 10-cm dishes with 10 ml of medium.

The transfected cells were analysed for the expression of d1EGFP protein (modified enhanced green fluorescent protein). All of the constructed plasmids expressed d1EGFP protein, which was detected by measuring the fluorescence using a flow cytometer. Because of the short half-life of the d1EGFP protein, it does not accumulate, and the expression of this protein reflects the presence of transcriptionally active plasmids in the cells. Becton-Dickinson FACSCalibur system was used. The volume of the Jurkat cell suspension was measured before each time-point (approximately after every 24 hour) and if the volume was less than 5 ml, the missing volume of medium was added. Depending on the cell suspension density the appropriate volume was

taken for measuring (1 or 2 ml) and replaced with the same amount of medium. This was later taken into consideration when the dilution was calculated.

For the first time-point, 293 cells from the 6-cm dish were suspended in 5 ml of medium for measuring. In every following time-point half of the cells
5 were taken from the 10-cm dish, suspended in 5 ml of medium and then measured. An appropriate volume was added to the rest of the cell suspension. For the first time-point, 3T6 cells from the 6-cm dish were suspended in 1 ml of trypsin, which was then inactivated with 100 µl of FCS. For every following time-point, cells from the 10-cm dish were suspended in 2 ml of trypsin. 1
10 ml of this suspension was treated as described previously. 9 ml of medium was added to the rest of the suspension. The analyzed cells were taken out of the incubator immediately before the measurement. The appropriate flow speed (500-1000 cells/sec) was determined before each time-point using cells transfected only with carrier DNA as a control. Three different parameters were
15 used to measure size, surface structure and fluorescence of the cells.

The results are presented as graphs in Figure 31. Cells transfected only with carrier DNA were used to measure the auto-fluorescence of the cell-line. 1% of this auto-fluorescence was considered as background fluorescence and was subtracted later from the d1EGFP fluorescence. The received data was
20 analyzed using Microsoft Excel program.

Percentages of the d1EGFP expressing cells were calculated using cells transfected with the carrier only as a negative control for background fluorescence. As shown in Figure 33, the two vectors were maintained in the cells with different kinetics.

25 The number of the d1EGFP expressing cells was calculated taking the dilutions into consideration using cells transfected with the carrier only as a negative control for background fluorescence. As seen from figure 53, the plasmids expressing EBNA-1 and carrying EBNA-1 specific multimeric binding sites are maintained very efficiently in the transfected cells. At day 1 after
30 transfection approximately 8×10^4 cells expressed EGFP. At day 8, in the case of maintenance vector (FREBNAAd1EGFP), the number of the plasmid positive d1EGFP expressing cells had increased ten times to 8×10^5 . With the plasmid

lacking EBNA-1 expression (FRE2d1EGFP) or having no EBNA-1 binding sites, the number of plasmid positive cells was retained or in many cases decreased. This fact reflects the mechanism for segregation/partitioning Epstein-Barr virus. Maintenance and segregation function by EBNA1 and EBNA-1 binding sites provides maintenance function to the plasmid if EBNA-1 is expressed and plasmid carries EBNA-1 binding sites. The same mechanism and the same components actually provide the segregation function to Epstein-Barr Virus in the latent phase of life-cycle.

Similar results were obtained also in human embryonic cell line 293 and mouse cell line 3T6 (Figure 34). As a control for the maintenance for 293 and 3T6 cells, s6HPV11 and 2wtFS, respectively, were used.

6.13 EXAMPLE 13: THE IMMUNOGENICITY OF GTU-MULTIGENE VECTORS

THE IMMUNOGENICITY OF GTU-1- MULTIGENE VECTORS

The immunogenicity of six different multi-gene vaccine constructs prepared in Example 12, i.e. GTU-1-RNT, GTU-1-TRN, GTU-1-RNT-CTL, GTU-1-TRN-CTL, GTU-1-TRN-optgag-CTL, and GTU-1-TRN-CTL-optgag vectors were tested in mice. The vectors were transformed into TOP10 or DH5alpha cells, and the MegaPreps were prepared using commercial Qiagen columns. Endotoxins were removed with Pierce Endotoxin Removal Gel.

The test articles were coated on 1 µm gold particles according to the instructions given by the manufacturer (Bio-Rad) with slight modifications. Balb/c mice were immunized with a Helios Gene Gun using a pressure of 400 psi and 0.5 mg gold/cartridge. Mice were immunized three times at weeks 0, 1, and 3. Mice were sacrificed two weeks after the last immunization.

Mice were divided into six test groups (5 mice/group), which received 3 x 1 µg DNA as follows:

- Group 1. GTU-1-RNT
- Group 2. GTU-1-TRN
- Group 3. GTU-1-RNT-CTL
- Group 4. GTU-1-TRN-CTL
- Group 5. GTU-1-TRN-optgag-CTL
- Group 6. GTU-1-TRN-CTL-optgag

Group 7. Control mice immunized with empty gold particles not loaded with DNA.

The humoral response was followed from tail-blood samples from each mouse. First pre-immunization sample was taken from anesthetized mice 5 before the first immunization was given. Second sample was taken from anesthetized mice before the third immunization. At sacrifice, whole blood sample was used for white blood cell counting, and serum was collected for humoral immunity tests.

The blood samples were tested for antibodies with ELISA using a 10 standard procedure. Nunc Maxi Sorp plates were coated with 100 ng of Nef, Rev, Tat, Gag, CTL or E2 proteins, blocked and sera at a dilution of 1:100 were added in duplicate wells for an overnight incubation. After washing, the plates were incubated for 2 hours with a diluted (1:500) secondary antibody, peroxidase conjugated anti-mouse IgG (DAKO). Color intensity produced from 15 the substrate (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) in phosphate-citrate buffer was measured at 405 nm using Labsystems ELISA-plate reader.

All vectors induced Nef antibodies in all mice, whereas none of the 20 mice showed E2, CTL or Rev antibodies (Fig. 35, 36, and Table 4). Some of the mice immunized with GTU-1-RNT or GTU-1-RNT-CTL also developed Tat 25 antibodies (Fig. 36 and Table 4). Furthermore, mice immunized with vectors containing the optgag sequence developed also Gag antibodies, but the construct GTU-1-TRN-optgag-CTL was a better antibody inducer than the construct GTU-1-TRN-CTL-optgag (Figure 37 and Table 4). The antibodies induced were mainly of the IgG1 class indicating a Th2 type of response usually seen with gene gun immunization. The antibody assays shown below were done from the sera collected when mice were sacrificed.

The results show that a multigene construct, expressing several HIV 30 genes as a fusion protein, can induce an immune response to most of the gene products. The orientation and order of the genes in the multigene and corresponding proteins in the fusion proteins affects the results, however, dramatically. Thus, a response against Tat was seen only when the Tat gene 35 was placed inside the fusion protein (vectors with RNT motif) and not when Tat was the amino terminal protein (vectors with the TRN motif). Response to the Gag proteins was seen only with the vector, where Gag was placed before the CTL containing a stretch of Th and CTL epitopes.

Table 4.

Immuno III A mice ELISA results (OD mean of five mice)						
Immunogen	Group number	Nef (own prot)	Tat	Rev	Gag	CTL
GTU-1-RNT	1	2.194	1.391	0.31	0.155	0.36
GTU-1-TRN	2	1.849	0.197	0.252	0.302	0.38
GTU-1-RNT-CTL	3	1.922	0.555	0.295	0.154	0.439
GTU-1-TRN-CTL	4	1.677	0.211	0.298	0.14	0.425
GTU-1-TRN-optgag-CTL	5	1.722	0.182	0.24	0.667	0.381
GTU-1-TRN-CTL-optgag	6	0.547	0.225	0.322	0.228	0.43
Controls	7	0.316	0.226	0.282	0.16	0.405
Immunogen	Group	Percent of Nef response	Tat response	Rev response	Gag response	CTL response
GTU-1-RNT	1	100	80	0	0	0
GTU-1-TRN	2	100	0	0	20	0
GTU-1-RNT-CTL	3	100	40	0	0	0
GTU-1-TRN-CTL	4	100	0	0	0	0
GTU-1-TRN-optgag-CTL	5	80	0	0	60	0
GTU-1-TRN-CTL-optgag	6	100	0	0	20	0

6.14. EXAMPLE 14: EXPRESSION OF HYBRID PROTEIN EXPRESSING NEF, REV AND TAT IN DIFFERENT COMBINATIONS (MULTIREG)

For the production of HIV multi-gene vectors, GTU-1 vector with a multi-cloning site (Figure 38A) was used as a backbone. Intact Nef, Rev and Tat coding sequences were amplified by the polymerase chain reaction (PCR) and attached to each other in various orders to multi-regulatory (multireg) antigen coding reading frames (Nef-Tat-Rev, Tat-Rev-Nef, Rev-Tat-Nef, Tat-Nef-Rev and Rev-Nef-Tat; Sequences Id. No. 1 to 5, respectively). These sequences were cloned to the Bsp119I and NotI sites of the GTU-1 vector.

Similarly, Nef protein expressing GTU-2 and GTU-3 vectors (Figure 38B and 38C; see also Figure 6B for NNV-2wt) were also used as backbones for the production of HIV multigene vectors. Additionally, the vector super6wt expressing destabilized enhanced green fluorescent protein or d1EGFP (super6wtd1EGFP; Figure 17 and Figure 38D) and plasmid utilizing the EBNA-1 protein and its binding sites (FREBNAd1EGFP; Figure 38E) were used as a Gene Transfer Unit (GTU) platform. For control "non-GTU" vectors, a regular cytomegalovirus (CMV) vector NNV-Rev expressing Rev and a plasmid EBNA-1 and E2BS containing d1EGFP plasmid (NNV-Rev and E2BSEBNAd1EGFP, respectively; Figures 38G and F) were used as backbones.

For the preparation of different GTU-2 and GTU-3 vectors (pNRT, pTRN, pRTN, pTNR and pRNT; and p2TRN and p2RNT; and p3RNT, Figures 39A-E, 39F-G and 39H, respectively), the Nef gene in vectors GTU-2Nef and GTU-3Nef was substituted by the respective multireg antigen using NdeI and Pst I sites. The sequence of the letters N(ef), R(ev) and T(at) in the name shows the position of respective coding sequences of the protein in the multi-gene. Also two vectors, which contain the IRES element placed into the SalI sites following either the multi-antigen or E2 coding sequences, were prepared (pTRN-iE2-GMCSF and pTRN-iMG-GMCSF, respectively; Figures 39I and J). The latter sequence, which controls the translation of the coding sequence of the mouse granulocyte-macrophage colony stimulating factor (GM-CSF), was cloned into the single BspTI site introduced with IRES.

Additionally, a set of the vectors, in which only immunodominant parts of the regulatory proteins were used for building up the polyproteins, were cloned into the Bsp119I and NotI sites of the GTU-1 (pMV1NTR, pMV2NTR, pMV1N11TR and pMV2N11TR; Figures 40A-D). In case of the pMV2 constructs, linkers that could be digested by intracellular proteases separate the regions of the multi-antigene derived from different regulatory proteins.

Further, GTU-1, GTU-2 and GTU-3 vectors, which express the structural proteins encoded by the gag gene or an artificial polyprotein composed by previously described CTL epitopes, were prepared. The coding sequences 10 were cloned as Bsp119I and Not I digested PCR fragments into the GTU-1 vector (pCTL = BNmCTL, pdgag = pBNdgag, psynp17/24 = pBNsynp17+24, poptyp17/24= pBNoptp17/24; Figures 41A-D), and transferred in a Nde I-Pac I fragment to the GTU-2 (p2mCTL and p2optp17/24; Figures 41E and F) and GTU-3 (p3mCTL and p3optp17/24; Figures 41G and H).

15 The coding segment designated as CTL (Sequence Id. No.10) contains fragments from pol and env regions involving many previously identified CTL epitopes. The codon usage is optimized so that only codons used frequently in human cells are involved. This coding sequence also contains a well-characterized mouse CTL epitope used in potency assay and an epitope for 20 recognition by anti-mouse CD43 antibody. Also, a dominant SIV p27 epitope was included for use in potency studies in macaques.

The dgag contains truncated p17 (start at 13 aa) +p24+p2+p7 (p1 and p6 are excluded) (Sequence Id. No. 11) of gag region of the Han2 isolate. The synp17/24 (Sequence Id. No. 12) codes for the p17+p24 polypeptide of the 25 Han2 HIV-1. The codon usage is modified to be optimal in human cells. Also, previously identified AU rich RNA instability elements were removed by this way. The optp17/24 coding (Sequence Id. No. 13) region is very similar to the synp17/24 with the exception that the two synonymous mutations made therein do not change the protein composition but remove a potential splicing 30 donor site.

Further, a set of the multi-HIV vectors, which contain both a multireg antigen and structural antigens as a single polyprotein, were created: pTRN-CTL,

- pRNT-CTL, pTRN-dgag; pTRN-CTL-dgag, pRNT-CTL-dgag, pTRN-dgag-CTL, pRNT-dgag-CTL, pTRN-optp17/24-CTL, pTRN-CTL-optp17/24, and pRNT-CTL-optp17/24; p2TRN-optp17/24-CTL, p2RNT-optp17/24-CTL, p2TRN-CTL-optp17/24, p2RNT-CTL-optp17/24, p2TRN-CTL-optp17/24-iE2-mGMCSF, and 5 p2RNT-CTL-optp17/24-iE2-mGMCSF; and p3TRN-CTL-optp17/24, p3RNT-CTL-optp17/24, p3TRN-CTL-optp17/24-iE2-mGMCSF, and p3RNT-CTL-optp17/24-iE2-mGMCSF, Figures 42A-T.

For cloning, as a first step the STOP codon was removed from the regulatory multi-antigen coding sequences. Then the structural antigen coding sequences were added by cloning into the NotI site at the end of the frame so that a NotI site was reconstituted. If both CTL and gag were added, the first antigen coding sequence was without the STOP codon. Generally, the 10 clonings were made in context of GTU-1 and for making the respective GTU-2 (p2...) and GTU-3 (p3...) vectors, the Nef gene in the plasmids GTU-2Nef and 15 GTU-2Nef was replaced using sites for NdeI and Pst I. However, the RNT-optp17/24-CTL antigen was built up directly in GTU-2 vector.

The HIV multi-antigen was cloned to the vectors super6wt1EGFP and FREBNAd1EGFP instead of the d1EGFP using sites for Eco105I and NotI (super6wt-RNT-CTL-optp17/24 and FREBNA-RNT-CTL-optp17/24; Figures 43V 20 and 42 U, respectively). If indicated, the IRES and mouse mGM-CSF were cloned into the GTU-2 and GTU-3 vectors behind the E2 coding sequence into the sites Mph1103I and Eco91I from pTRN-iE2-mGMCSF (cut out using same 25 restrictionases).

Finally, "non-GTU" control vector E2BSEBNA-RNT-CTL-optp17/24 25 (Figure 42W) for the system utilizing EBNA-1 (contains EBNA-1 expression cassette with E2 binding sites) was made in a similar way as the FREBNA-RNT-CTL-optp17/24. The regular CMV vector pCMV-RNT-CTL-optp17/24 expressing the multi HIV antigen (Figure 42D) was made by cloning the multi-HIV coding fragment from respective GTU-1 vector using sites for NdeI and Pst I.

6.15. EXAMPLE 15: EXPRESSION PROPERTIES OF THE MULTIREG ANTIGENS CARRYING ONLY IMMUNODOMINANT REGIONS OF THE REGULATORY PROTEINS.

1. Intracellular localization of the MultiREG antigens

The intracellular localization of MultiREG antigens expressed by the vectors of the invention was studied by *in situ* immunofluorescence in RD cells using monoclonal antibodies against Nef, Rev and Tat proteins essentially as described in Example 4. The results are summarized in Table 5 and illustrated in figure 45. All antigens that are comprised of intact Nef, Rev and Tat proteins showed exclusive localization in cytoplasm. The aberrant protein initially designed as N(ef)T(at)R(ev), which has a frame-shift before the Rev sequence, showed only the nuclear localization. MultiREG antigens carrying truncated sequences of the regulatory proteins were localized in cytoplasm. In this cases distinct structures like "inclusion bodies" were frequently observed. The same was true for antigens, which carried the protease sites expressed from pMV2 vectors. However in these cases the proteins in nucleus were also detected (Figure 45).

Table 5. Intracellular localization in multireg antigens

Construct	anti-Nef	anti-Rev	anti-Tat
empty	negative	negative	negative
GTU-1			
pTRN	strong staining in cytoplasm	good staining in cytoplasm	positive staining in cytoplasm
pNTR	strong staining in nucleus, nucleolus	negative	positive staining in nucleus
pRNT	strong staining in cytoplasm	good staining in cytoplasm	good staining in cytoplasm
pNRT	strong, cytoplasmic	good staining in cytoplasm	good staining in cytoplasm
pRTN	strong, cytoplasmic	good staining in cytoplasm	positive staining in cytoplasm
pTNR	strong, cytoplasmic	good staining in cytoplasm	good staining in cytoplasm
pMV1NTR	strong, cytoplasmic	cytoplasmic+inclusions	cytoplasmic+inclusion
pMV1N11	strong cytoplasmic+inclusions	cytoplasmic+inclusions	cytoplasmic+inclusion
TR			
pMV2NTR	inclusions in nuclei and in cytopi.	inclusions in nuclei and cytoplasm	inclusions in nuclei and cytoplasm
pMV2N11	only inclusions, in nuclei and in cytopi	only inclusions in nuclei and in cytopi	only inclusions in nuclei and in cytopi.

The intracellular localization of dgag and p17+p24 proteins was also analyzed in RD cells by immunofluorescence with monoclonal anti p24 antibodies. In accordance with the Western blot results in Jurkat cells, the dgag could not be detected. However, the p17/24 protein showed localization in 5 plasma membranes (Figure 45). The localization of CTL protein was not analyzed, because no suitable antibody was available.

6.2 EXAMPLE 15: ANALYSIS OF VECTORS ENCODING RECOM-
10 BINANT GAG ANTIGENS AND CYTOTOXIC T-CELL EPITOPE (CTL)
FROM POL

6.2.1. EXPRESSION

Analysis of expression of the vectors expressing CTL cds or proteins from the gag region were performed by western blot. As seen on Figure 46A 15 and 46B, the CTL and dgag expression was clearly demonstrated in Cos-7 cells as predicted size proteins (25kD and 47kD, respectively). The co-transfection of the Nef, Rev and Tat significantly enhanced the expression of the dgag protein. We interpret this as a result of REV protein action on the GAG mRNA expression. We also tried to express the dgag protein from GTU-1 20 vector in Jurkat cells, but we failed to detect any signal (Figure 46C). The analysis of the codon usage showed that wt GAG sequence had not optimal codon usage for human cells. When the codon usage was optimized (constructs psynp17/24 and poptyp17/24), strong p17+p24 (40kD) protein expression was detected in Jurkat cells (Figure 46C and 46D).

25 6.2.2. INTRACELLULAR LOCALIZATION

For dgag and p17+p24 proteins, the intracellular localization was also analyzed in RD cells by immunofluorescence with anti p24 Mab. Similar to the western blot results in Jurkat cells, the dgag could not be detected. The p17/24 protein showed localization in plasma membranes (Figure 47). The localization 30 of CTL protein was not analyzed caused by lacking of suitable antibody.

6.16 EXAMPLE 16: MULTIREG+STRUCTURAL PROTEINS AS MULTI HIV ANTIGEN EXPRESSION

5 As next step, the expression of the MultiHIV antigens consisting of both, regular multigene together with gag encoded protein and/or CTL multiepitope as single polypeptide was analysed. On Figure 48, the Western blot shows the expression of several multiHIV-antigenes expressing vectors transfected to the Cos-7 cells. It is clearly seen that the expression levels of all
10 regulatory+structural multi-antigenes are significantly lower than of the RNT or TRN proteins. All tested MultiHIV antigenes migrate in the gel as distinct bands near the position of predicted size (73kD for multireg+CTL; 95kD for multireg+dgag and 120kD for multireg+CTL+dgag). Similar to the RNT and TRN, the RNT-CTL migrates more slowly than TRN-CTL. Also, in cases of both TRN
15 and RNT constructs, the MultiREG-CTL-dgag combination showed higher expression level than MultiREG-dgag-CTL.

More detailed analysis of the multiHIV antigens was performed in Jurkat cells. For this reason, most of the constructed MultiHIV antigenes (multireg+structural), included the MultiREG+CTL+optp17/24 (with predicted size
20 113kD) were analyzed by Western blotting using antibodies against different parts of the antigen. The results are presented on Figure 49 are principally similar to those were reported in previous section in case of Cos-7 cells. As it was seen in the previous experiments, the dgag containing multi-antigenes express very low levels of the hybrid protein in Jurkat cells. The expression
25 from the vector pTRNdgag was undetectable on all blots. In lanes loaded material from cells transfected with other dgag containing antigen expression vectors, very faint signals only on the Nef Mab hybridized blot were detected at positions of predicted sizes. In contrast, if the dgag part is replaced with the codon optimized p17/24, the expression level increase was observed. Because the TRN-CTL-optp17/24 and RNT-optp17/24 were initially chosen for further analysis, the expression of the antigens was analyzed from all GTU
30 vectors containing these expression cassettes. Also, the E2 protein expression

from these plasmids was analyzed. The results are illustrated on Figure 50. There are no big differences between the vectors in expression levels of both multi-antigene and the E2 protein. The E2 expression level is not significantly influenced by presence of IRES element followed mouse GM-CSF gene in the 5 plasmid, translated from the same mRNA as the E2.

6.17. EXAMPLE 17: MAINTENANCE OF EXPRESSION OF ANTIGEN

The maintenance of the plasmid in a population of dividing cells was proved using the green fluorescent protein and Nef protein as markers. The 10 maintenance of the expression of the RNT-CTL-optp17/24 antigen produced from different GTU or non-GTU vectors was also analyzed. Specifically, GTU-1 (p RNT-CTL-optp17/24), GTU-2 (p2 RNT-CTL-optp17/24), GTU-3 (p3 RNT-CTL-optp17/24), super6wt (super6wt-RNT-CTL-optp17/24) vectors each utilize the E2 protein and its binding sites for the plasmid maintenance activity. In this 15 experiment, also EBNA-1 and its binding site utilizing GTU vector FREBNA-RNT-CTL-optp17/24 was included. As negative controls, "non-GTU" plasmid containing a mixed pair of the EBNA-1 expression cassette together with E2 binding sites (E2BSEBNA- RNT-CTL-optp17/24) was used. Also, regular CMV expression vector pCMV- RNT-CTL-optp17/24 was used.

20 Jurkat cells were transfected with equimolar amounts of the plasmids and the antigen expression was studied at 2 and 5 days post-transfection using a monoclonal anti-Nef antibodies. Transfection with carrier DNA only was used as a negative control. The results are presented in Figure 51.

As it seen from figure 51, the expression is detectable only from 25 GTU vectors at the second time-point. The antigen expression from the FREBNA- RNT-CTL-optp17/24 was lower at both time-points, because, unlike E2, the EBNA-1 does not have transcription activation ability.

Also the intracellular localization of the multireg+structural polyproteins was studied by *in situ* immunofluorescence analysis in RD cells essentially as described in Example 4. The results are presented in figure 52.

In all cases localization only in cytoplasm was detected using either monoclonal anti-Nef or anti-p24 antibodies. In accordance with Western blot data, the expression level of optp17/24 containing proteins was much stronger than dgag fragment containing antigens.

All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

5 Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims along with the full scope of equivalents to which such claims
10 are entitled.

WHAT IS CLAIMED IS:

1. An expression vector comprising:
 - (a) a DNA sequence encoding a nuclear-anchoring protein operatively linked to a heterologous promoter, said nuclear-anchoring protein comprising (i) a DNA binding domain which binds to a specific DNA sequence, and (ii) a functional domain that binds to a nuclear component, or a functional equivalent thereof; and
 - (b) a multimerized DNA sequence forming a binding site for the nuclear anchoring protein,
wherein said vector lacks a papilloma virus origin of replication.
2. The vector of claim 1, wherein said vector lacks an origin of replication functional in a mammalian cell.
3. The vector of claim 1 or 2, wherein said nuclear component is mitotic chromatin, the nuclear matrix, nuclear domain 10 (ND10), or nuclear domain POD.
4. The vector of claim 1 or 2, wherein said nuclear anchoring-protein is a chromatin-anchoring protein, and said functional domain binds mitotic chromatin.
5. The vector of claim 1 or 2, wherein said nuclear-anchoring protein contains a hinge or linker region.
6. The vector of claim 1 or 2, wherein said nuclear-anchoring protein is a natural protein of eukaryotic, prokaryotic, or viral origin.
7. The vector of claim 6, wherein said natural protein is of viral origin.
8. The vector of claim 6, wherein said nuclear-anchoring protein is a natural protein of eukaryotic origin.
9. The vector of claim 1 or 2, wherein said nuclear-anchoring protein is that of a papilloma virus or an Epstein-Barr virus.
10. The vector of claim 9, wherein said nuclear-anchoring protein is the E2 protein of Bovine Papilloma Virus type 1 or Epstein-Barr Virus Nuclear Antigen 1.

11. The vector of claim 10, wherein said nuclear-anchoring protein is the E2 protein of Bovine Papilloma Virus type 1.
12. The vector of claim 1 or 2, wherein said nuclear-anchoring protein is a High Mobility Group protein.
- 5 13. The vector of claim 1 or 2, wherein said nuclear-anchoring protein is a non-natural protein.
14. The vector of claim 13, wherein said nuclear-anchoring protein is a recombinant protein, a fusion protein, or a protein obtained by molecular modeling techniques.
- 10 15. The vector of claim 14, wherein said recombinant protein, fusion protein, or protein obtained by molecular modeling techniques contains any combination of a DNA binding domain which binds to said specific DNA sequence and a functional domain which binds to a nuclear component, wherein said functional domain which binds to a nuclear component is that of a papilloma virus, an Epstein-Barr-Virus, or a High Mobility Group protein.
- 15 16. The vector of claim 15, wherein said recombinant protein, fusion protein, or protein obtained by molecular modeling techniques contains any combination of a DNA binding domain which binds to said specific DNA sequence and a functional domain which binds to a nuclear component, wherein said functional domain which binds to a nuclear component is that of E2 protein of Bovine Papilloma Virus type 1, Epstein-Barr Virus Nuclear Antigen 1, or a High Mobility Group protein.
- 20 17. The vector of claim 1 or 2, wherein said vector further comprises one or more expression cassettes of a DNA sequence of interest.
- 25 18. The vector of claim 17, wherein said DNA sequence of interest is that of an infectious pathogen.
19. The vector of claim 18, wherein said infectious pathogen is a virus.
20. The vector of claim 19, wherein said virus is selected from the group consisting of Human Immunodeficiency Virus (HIV), Herpex Simplex Virus (HSV), Hepatitis C Virus, Influenzae Virus, and Enterovirus.
- 30 21. The vector of claim 18, wherein said DNA sequence of interest is that of a bacterium.

22. The vector of claim 21, wherein said bacterium is selected from the group consisting of Chlamydia trachomatis, Mycobacterium tuberculosis, and Mycoplasma pneumonia.
23. The vector of claim 21, wherein said bacterium is Salmonella.
- 5 24. The vector of claim 17, wherein said DNA sequence of interest is that of a fungal pathogen.
25. The vector of claim 24, wherein said fungal pathogen is Candida albicans.
- 10 26. The vector of claim 20, wherein said DNA sequence of interest is of HIV origin.
27. The vector of claim 26, wherein said DNA sequence of interest encodes a non-structural regulatory protein of HIV.
28. The vector of claim 27, wherein said non-structural regulatory protein of HIV is Nef, Tat or Rev.
- 15 29. The vector of claim 28, wherein said nonstructural regulatory protein of HIV is Nef.
30. The vector of claim 17, wherein said DNA sequence of interest encodes a structural protein of HIV.
31. The vector of claim 30, wherein said DNA sequence of interest is the gene encoding HIV gp120/gp160.
- 20 32. The vector of claim 17, wherein a first said expression cassette comprises a DNA sequence of interest which encodes Nef, Tat or Rev, and wherein a second said expression cassette comprises a DNA sequence of interest which encodes Nef, Tat or Rev.
- 25 33. The vector of claim 17, wherein a first said expression cassette comprises a DNA sequence of interest which encodes Nef, Tat or Rev, and wherein a second said expression cassette comprises a DNA sequence of interest which encodes a structural protein of HIV.
34. The vector of claim 17, wherein the DNA sequence of interest encodes a protein associated with cancer.

35. The vector of claim 17, wherein the DNA sequence of interest encodes a protein associated with immune maturation, regulation of immune responses, or regulation of autoimmune responses.
36. The vector of claim 35, wherein said protein is APECED.
- 5 37. The vector of claim 17, wherein the DNA sequence of interest is the Aire gene.
38. The vector of claim 17, wherein the DNA sequence of interest encodes a protein that is defective in any hereditary single gene disease.
- 10 39. The vector of claim 17, wherein the DNA sequence of interest encodes a macromolecular drug.
40. The vector of claim 39, wherein the DNA sequence of interest encodes a cytokine.
41. The vector of claim 40, wherein said cytokine is an interleukin selected from the group consisting of IL1, IL2, IL4, IL6 and IL12.
- 15 42. The vector of claim 40, wherein the DNA sequence of interest encodes an interferon.
43. The vector of claim 17, wherein said DNA sequence of interest encodes a biologically active RNA molecule.
44. The vector of claim 43, wherein said biologically active RNA molecule is selected from the group consisting of inhibitory antisense and ribozyme molecules.
- 20 45. The vector of claim 44, wherein said inhibitory antisense or ribozyme molecules antagonize the function of an oncogene.
46. The vector of claim 17 for use as a medicament.
- 25 47. The vector of claim 17 for use as a carrier vector for a gene, genes, or a DNA sequence or DNA sequences of interest, such as a gene, genes, or a DNA sequence or DNA sequences encoding a protein or peptide of an infectious agent, a therapeutic agent, a macromolecular drug, or any combination thereof.
- 30 48. The vector of claim 17 for use as a medicament for treating inherited or acquired genetic defects.

49. The vector of claim 17 for use as a therapeutic DNA vaccine against an infectious agent.
50. The vector of claim 17 for use as a therapeutic agent.
51. A vector of claim 17 for the use for production of a protein encoded by said DNA sequence of interest in a cell or an organism.
52. A vector of claim 17 for the use for production of a therapeutic macromolecular agent *in vivo*.
53. A method for providing a protein to a subject, said method comprising administering to the subject the vector of claim 1 or 2, wherein said vector (i) further comprises a second DNA sequence encoding the protein to be provided to the subject, which second DNA sequence is operably linked to a second promoter, and (ii) does not encode Bovine Papilloma Virus protein E1, and wherein said subject does not express Bovine Papilloma Virus protein E1.
54. A method for inducing an immune response to a protein in a subject, said method comprising administering to the subject the vector of claim 1 or 2, wherein said vector (i) further comprises a second DNA sequence encoding said protein, which second DNA sequence is operably linked to a second promoter, and (ii) does not encode Bovine Papilloma Virus protein E1, and wherein said subject does not express Bovine Papilloma Virus protein E1.
55. A method for treating an infectious disease in a subject in need of said treatment, said method comprising administering to said subject a therapeutically effective amount of the vector of claim 17, wherein said DNA sequence of interest encodes a protein comprising an immunogenic epitope of an infectious agent.
56. A method for treating an inherited or acquired genetic defect in a subject in need of said treatment, said method comprising: administering to said subject a therapeutically effective amount of the vector of claim 17, wherein said DNA sequence of interest encodes a protein which is affected by said inherited or acquired genetic defect.
57. A method for expressing a DNA sequence in a subject, said method comprising administering the vector of claim 17 to said subject.

58. The method of claim 55, 56, or 57, wherein said vector does not encode Bovine Papilloma Virus protein E1, and wherein said subject does not express Bovine Papilloma Virus protein E1.

59. A method for the preparation of a vector of claim 1 or 2 comprising:

- 5 (a) cultivating a host cell containing said vector; and
- (b) recovering the vector.

60. The method of claim 59, further comprising before step (a) a step of transforming said host cell with said vector.

61. The method of claim 59, wherein said host cell is a prokaryotic cell.

10 62. The method of claim 59, wherein said host cell is an Escherichia coli.

63. A host cell, characterized by containing the vector of claim 1 or 2.

64. A host cell, characterized by containing the vector of claim 17.

65. The host cell of claim 63, wherein said host cell is a bacterial cell.

15 66. The host cell of claim 63, wherein said host cell is a mammalian cell.

67. A carrier vector containing the vector of claim 1 or 2.

68. A pharmaceutical composition comprising the vector of claim 17 and a suitable pharmaceutical carrier.

20 69. A DNA vaccine containing the vector of claim 17.

70. A DNA vaccine containing the vector of claim 18.

71. A DNA vaccine containing the vector of claim 19.

72. A DNA vaccine containing the vector of claim 21.

73. A DNA vaccine containing the vector of claim 24.

25 74. A gene therapeutic agent containing the vector of claim 17.

75. A method for the preparation of the DNA vaccine of claim 69, said method comprising combining the vector of claim 17 with a suitable pharmaceutical vehicle.

30 76. A method for the preparation of the DNA vaccine of claim 69, said method comprising combining the vector of claim 18 with a suitable pharmaceutical vehicle.

77. A method for the preparation of the DNA vaccine of claim 69, said method comprising combining the vector of claim 19 with a suitable pharmaceutical vehicle.

78. A method for the preparation of the DNA vaccine of claim 69, said 5 method comprising combining the vector of claim 21 with a suitable pharmaceutical vehicle.

79. A method for the preparation of the DNA vaccine of claim 69, said method comprising combining the vector of claim 24 with a suitable pharmaceutical vehicle.

10 80. A method for the preparation of the agent of claim 74, said method comprising combining the vector of claim 17 with a suitable pharmaceutical vehicle.

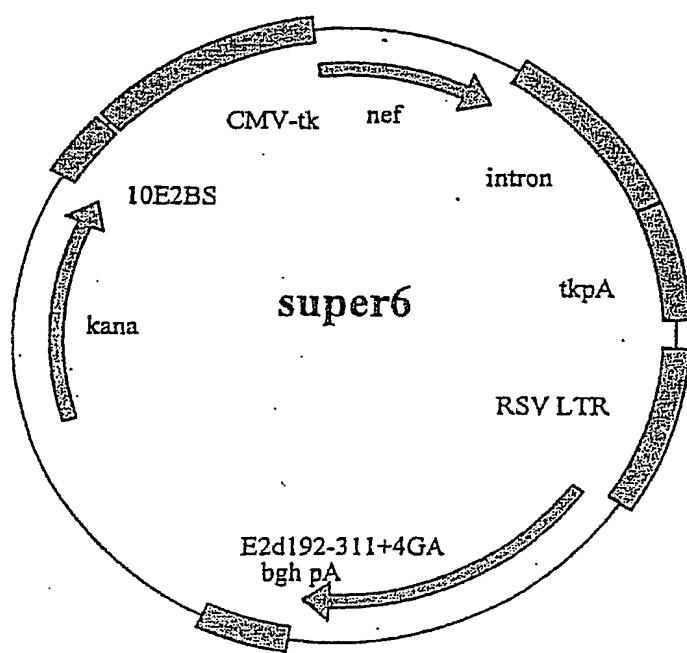


FIG. 1

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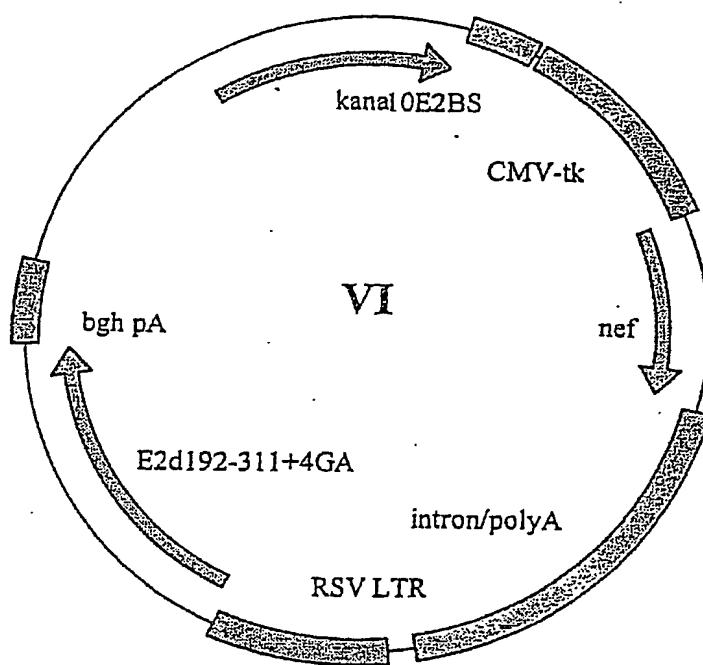


FIG. 2

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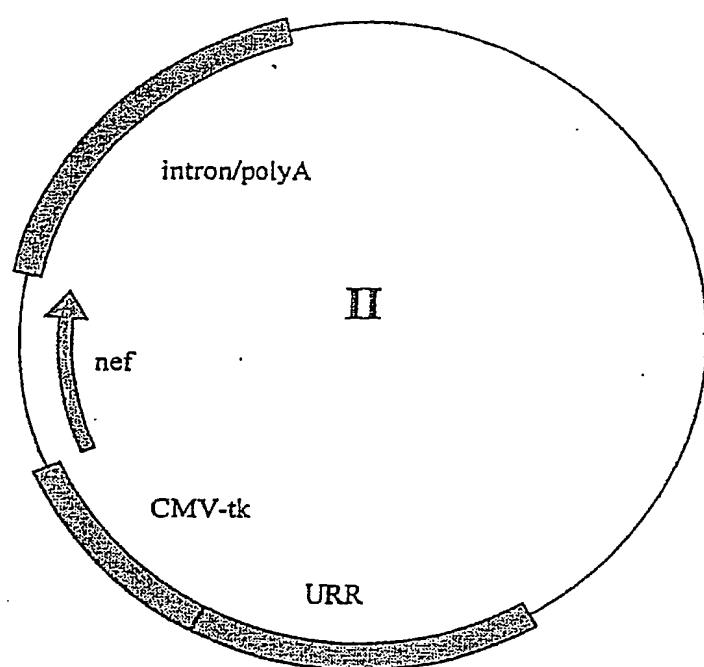


FIG. 3

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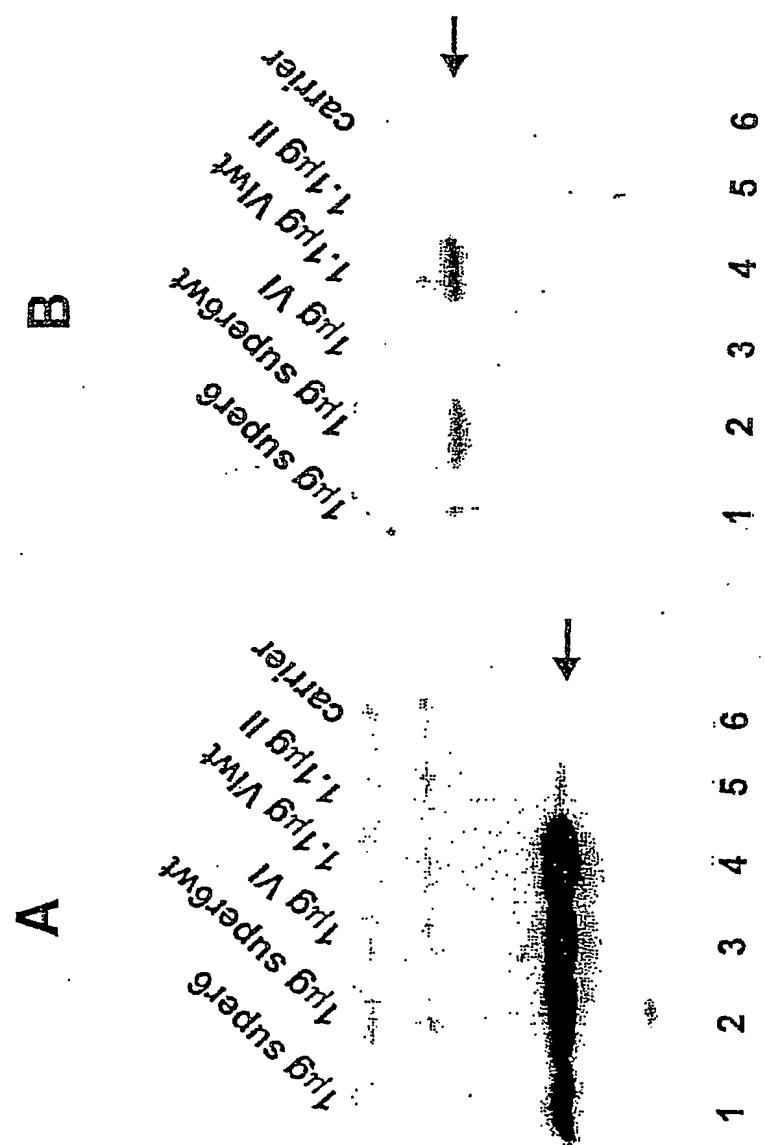


FIG. 4

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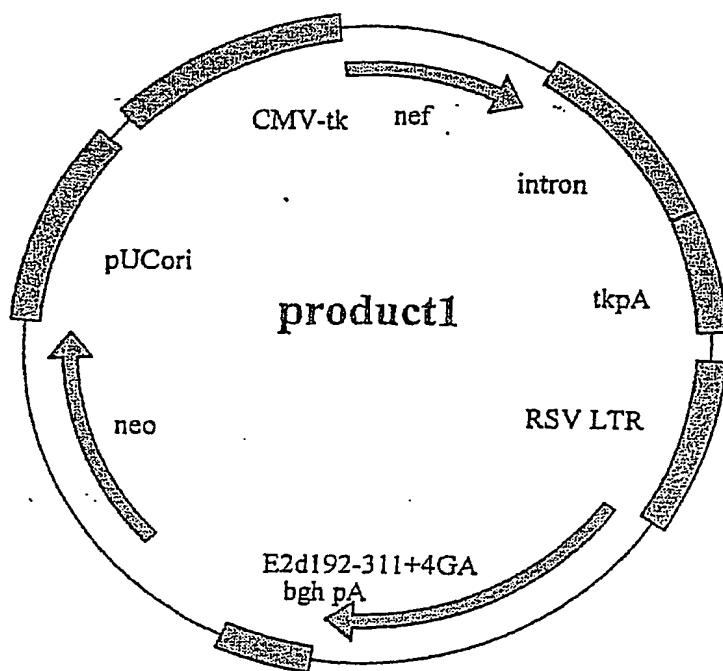


FIG. 5

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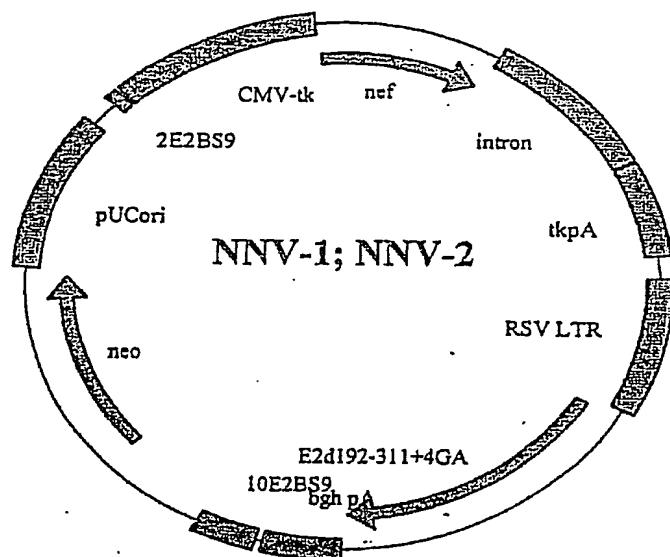


FIG. 6A

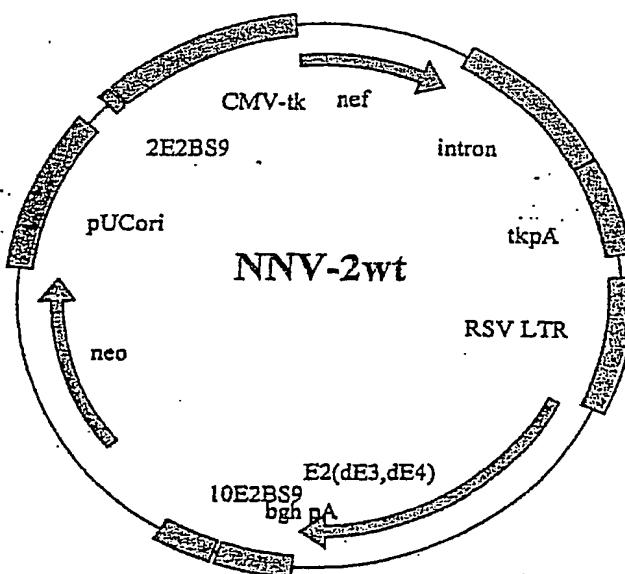


FIG. 6B

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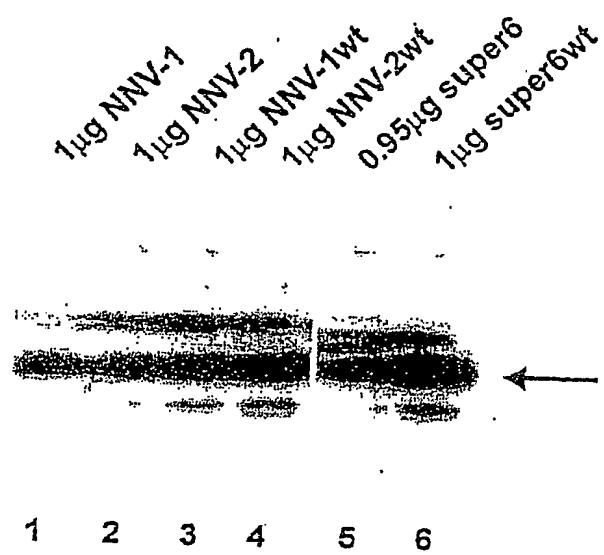


FIG. 7

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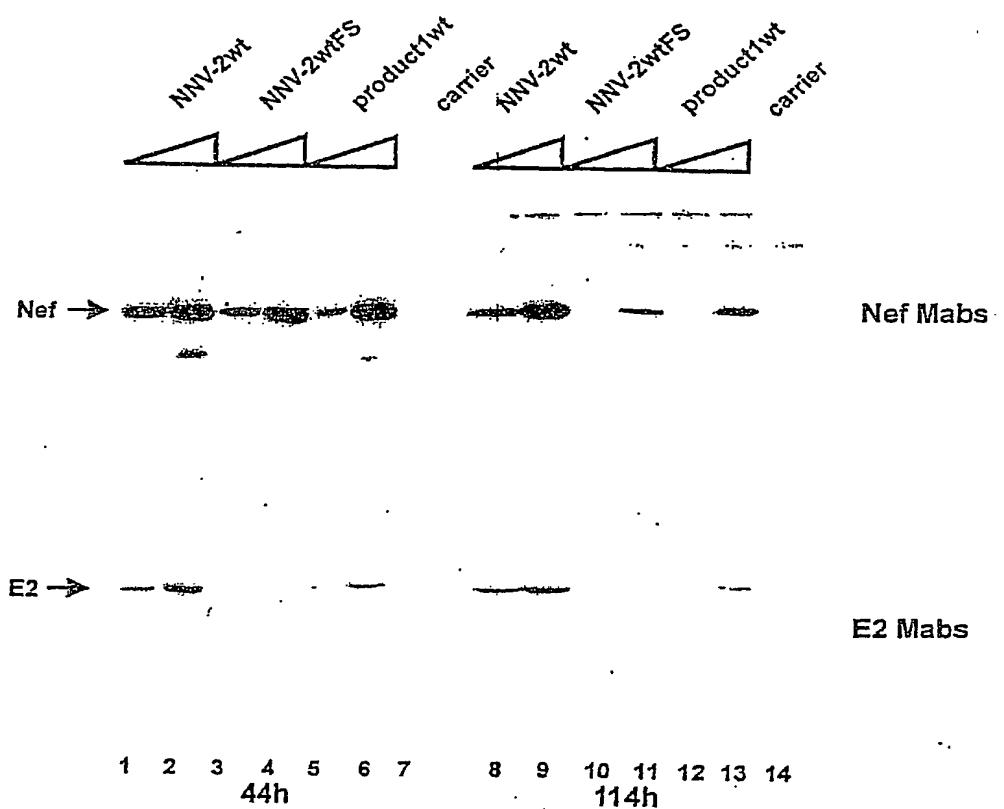


FIG. 8

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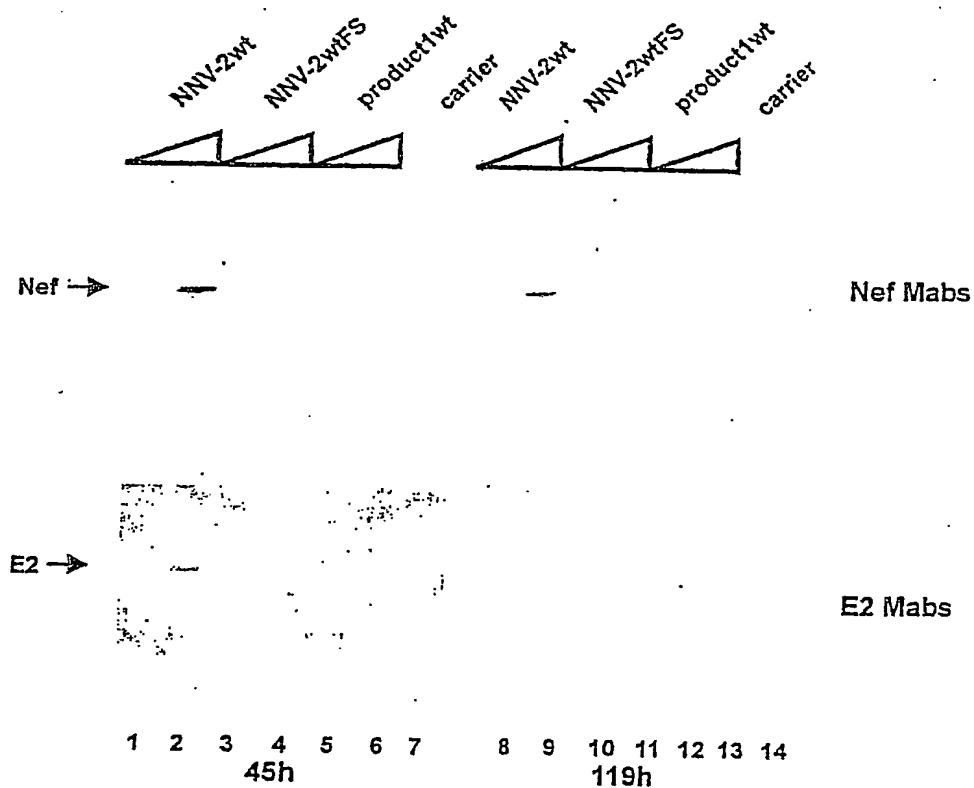


FIG. 9

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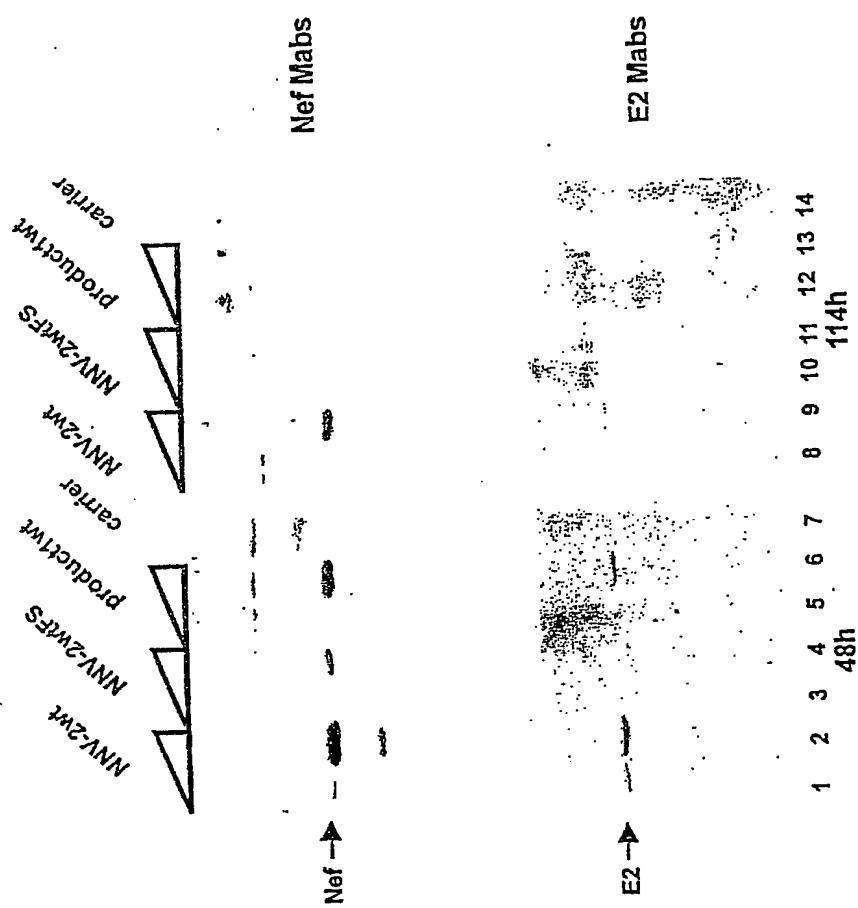


FIG. 10

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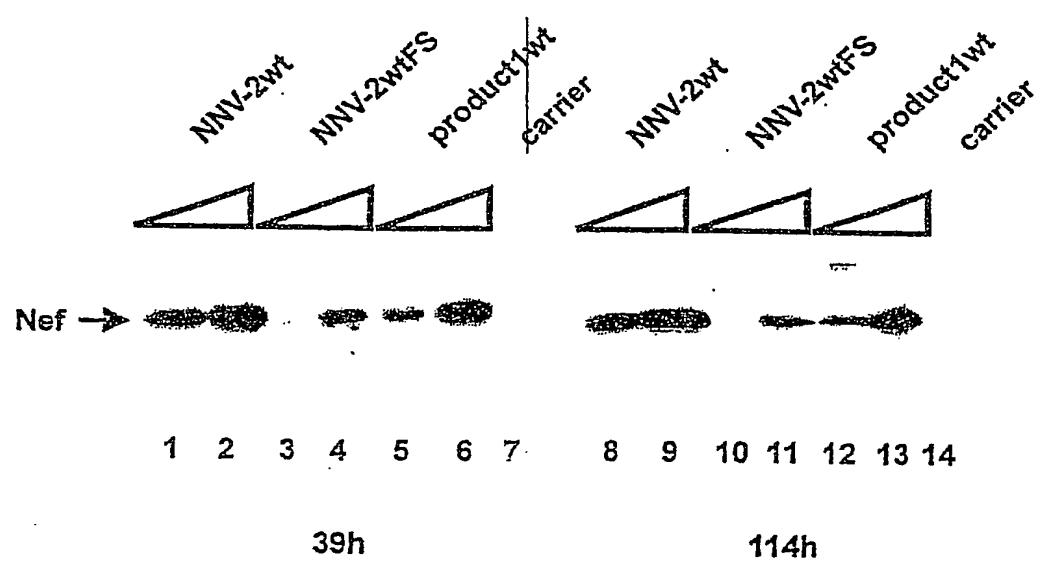


FIG. 11

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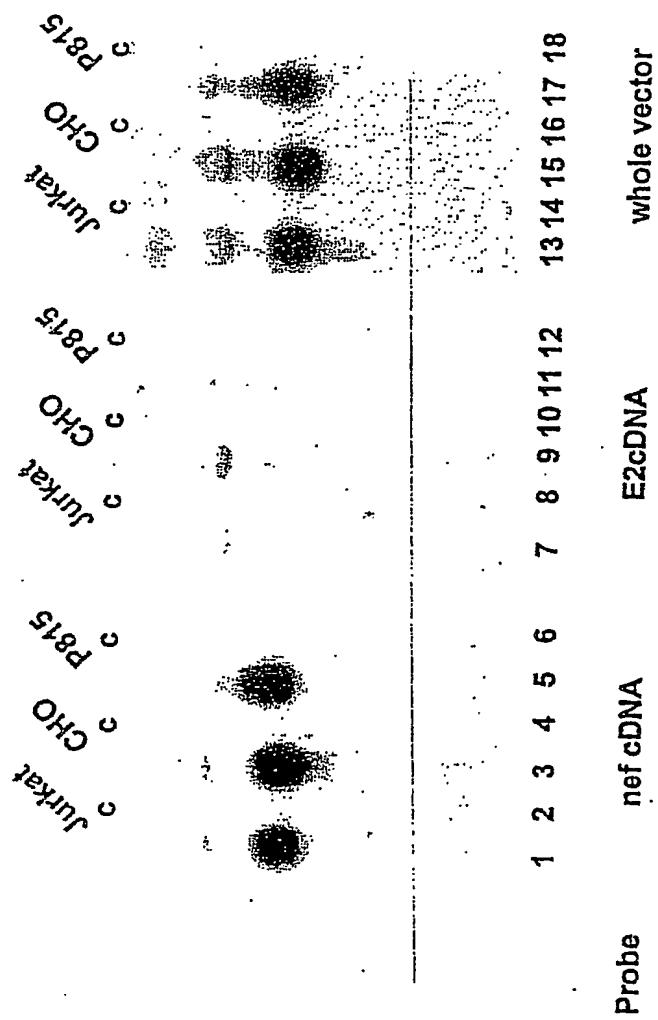


FIG. 12

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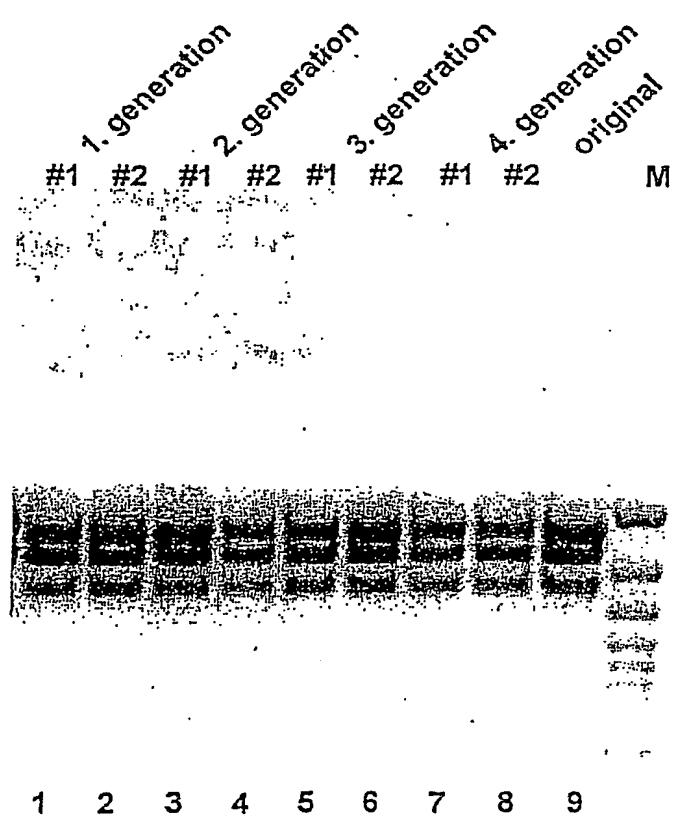


FIG. 13

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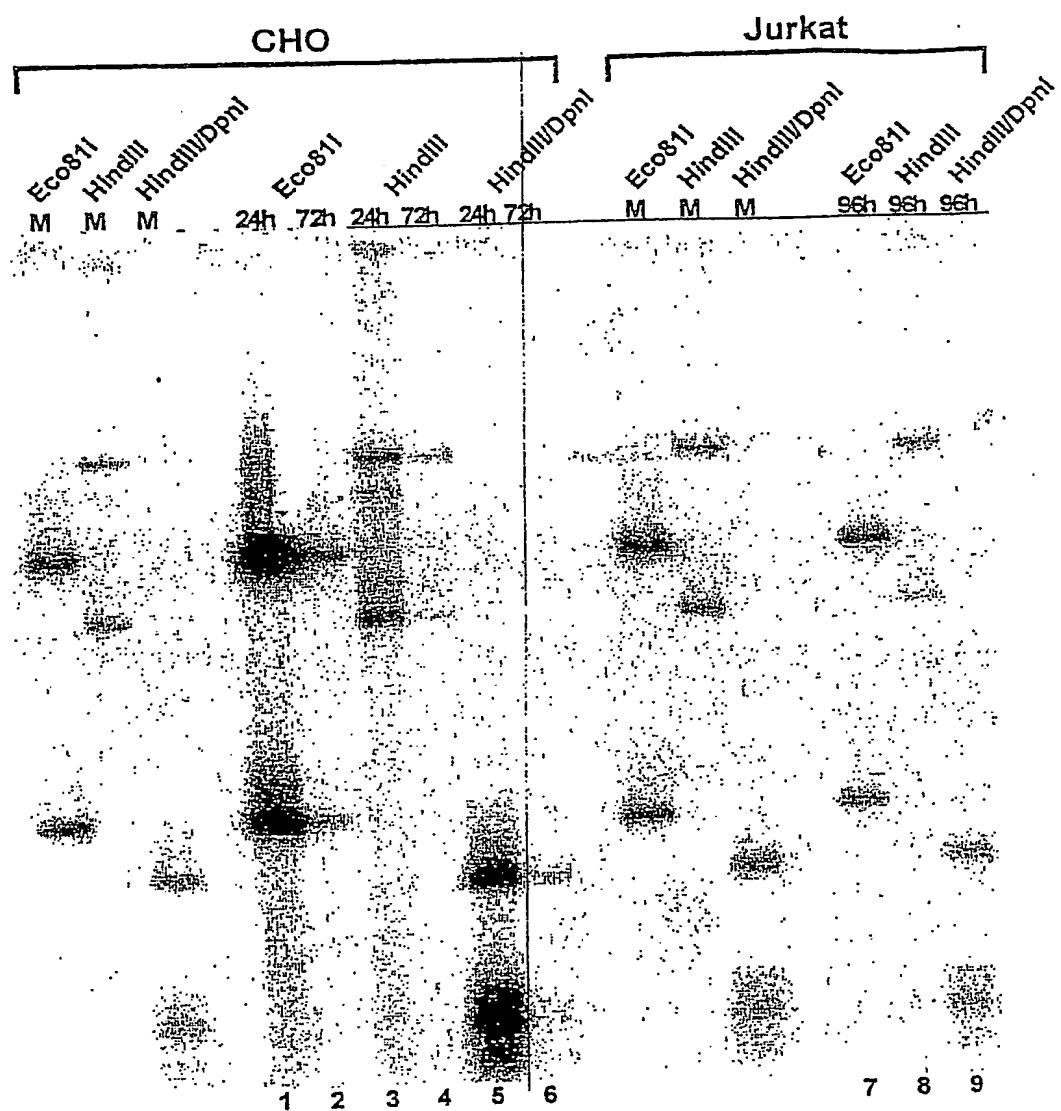


FIG. 14

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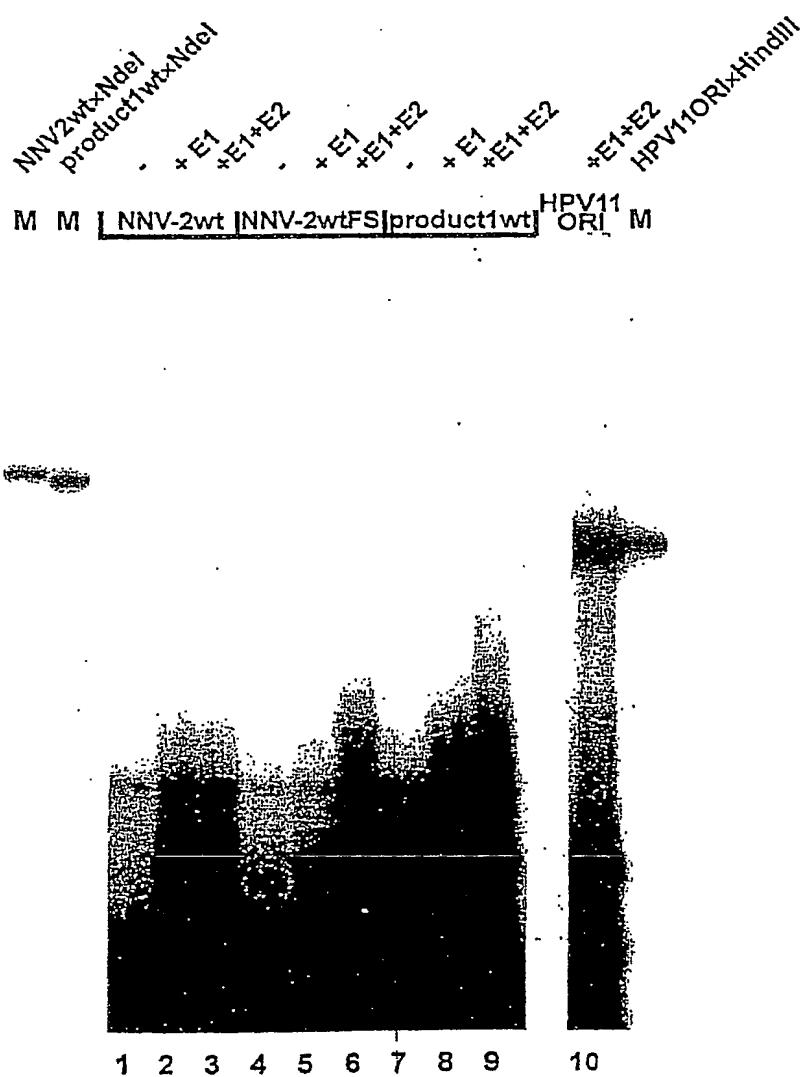


FIG. 15

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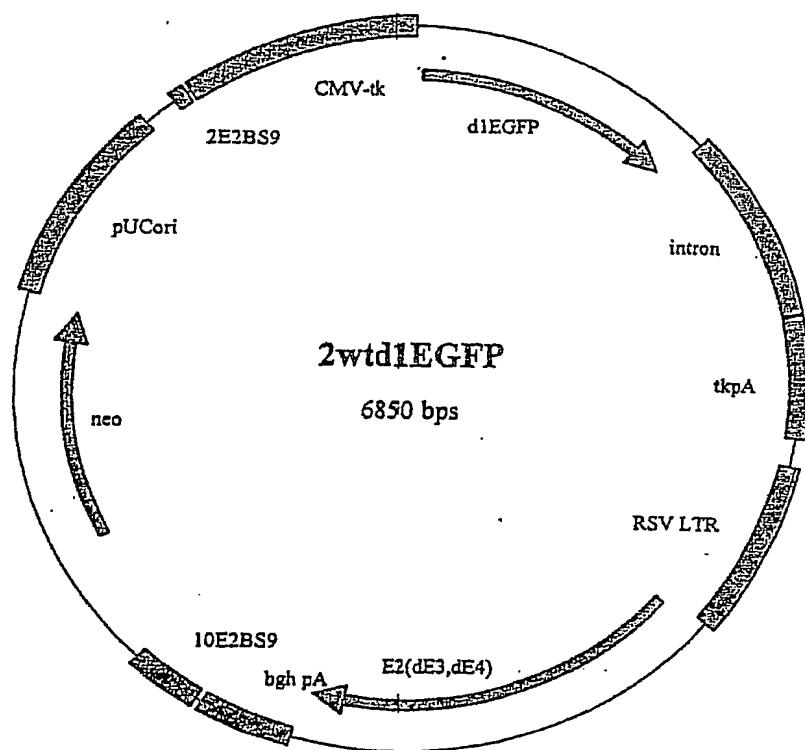


FIG. 16

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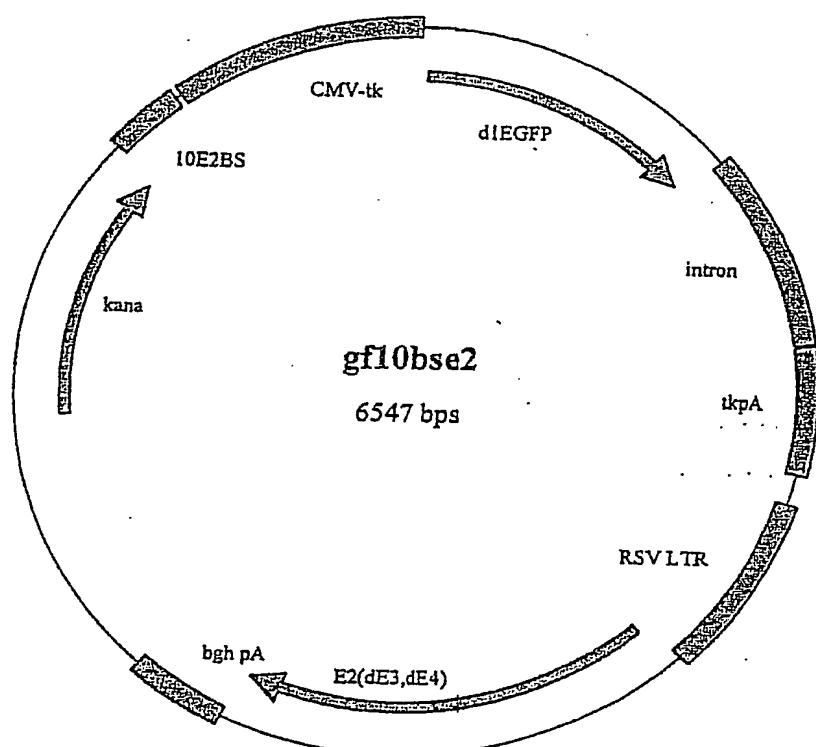
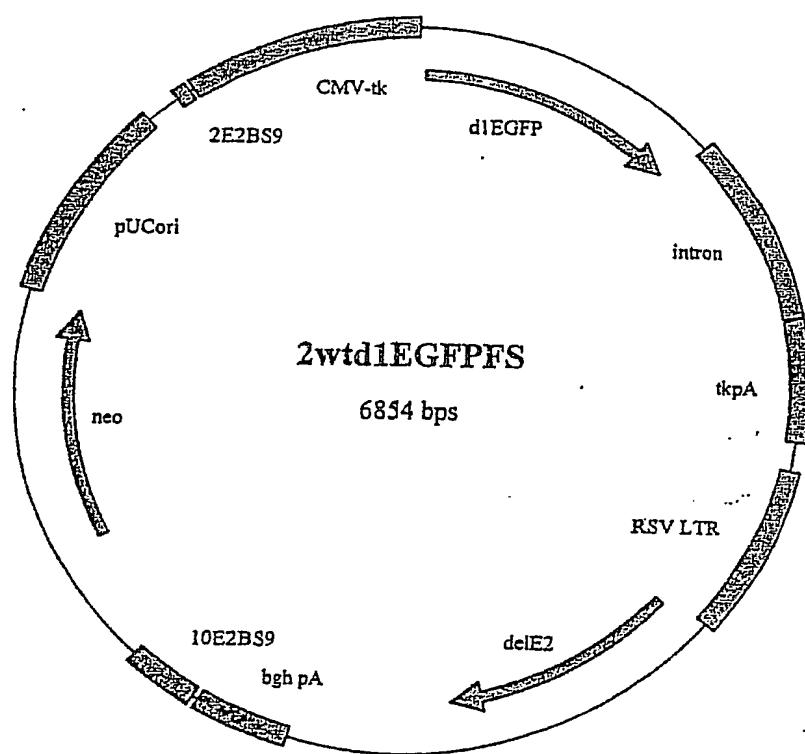
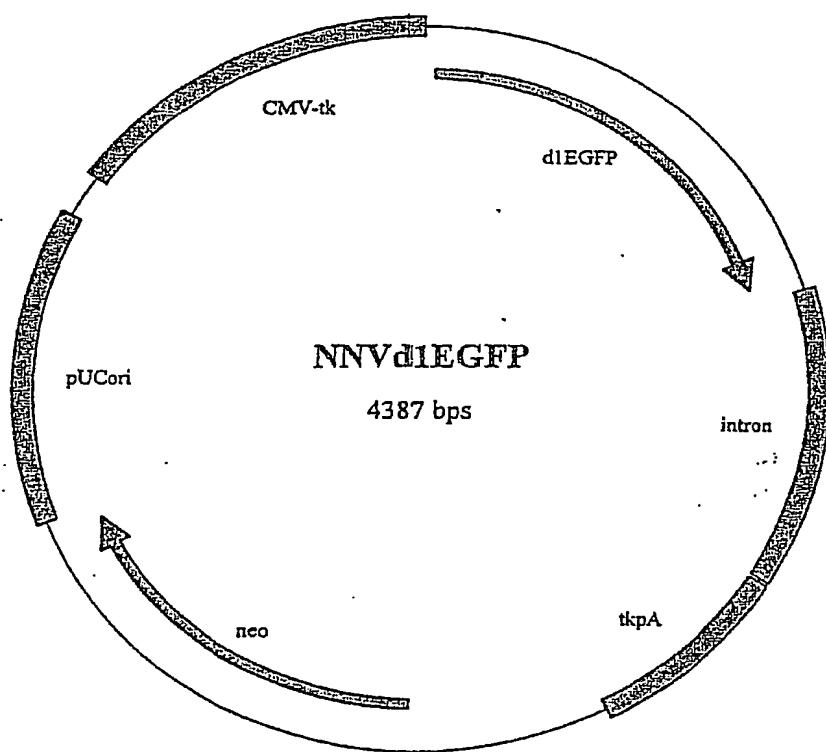


FIG. 17

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**FIG. 18**

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**FIG. 19**

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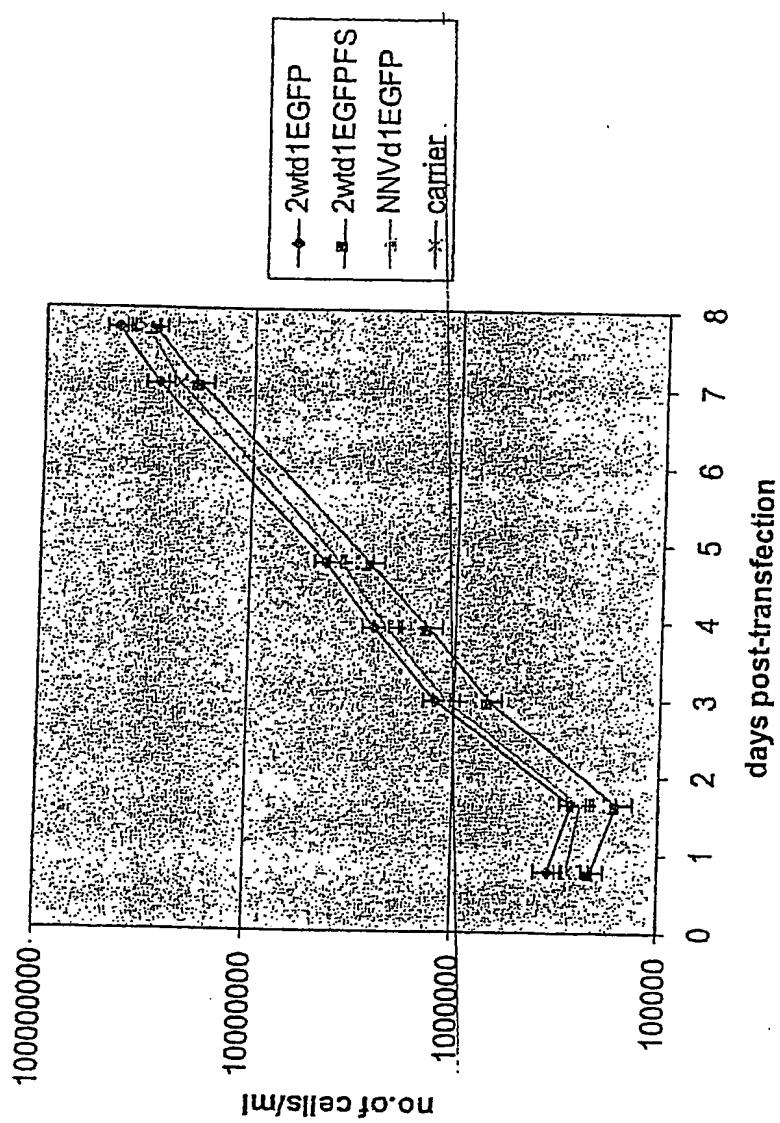


FIG. 20

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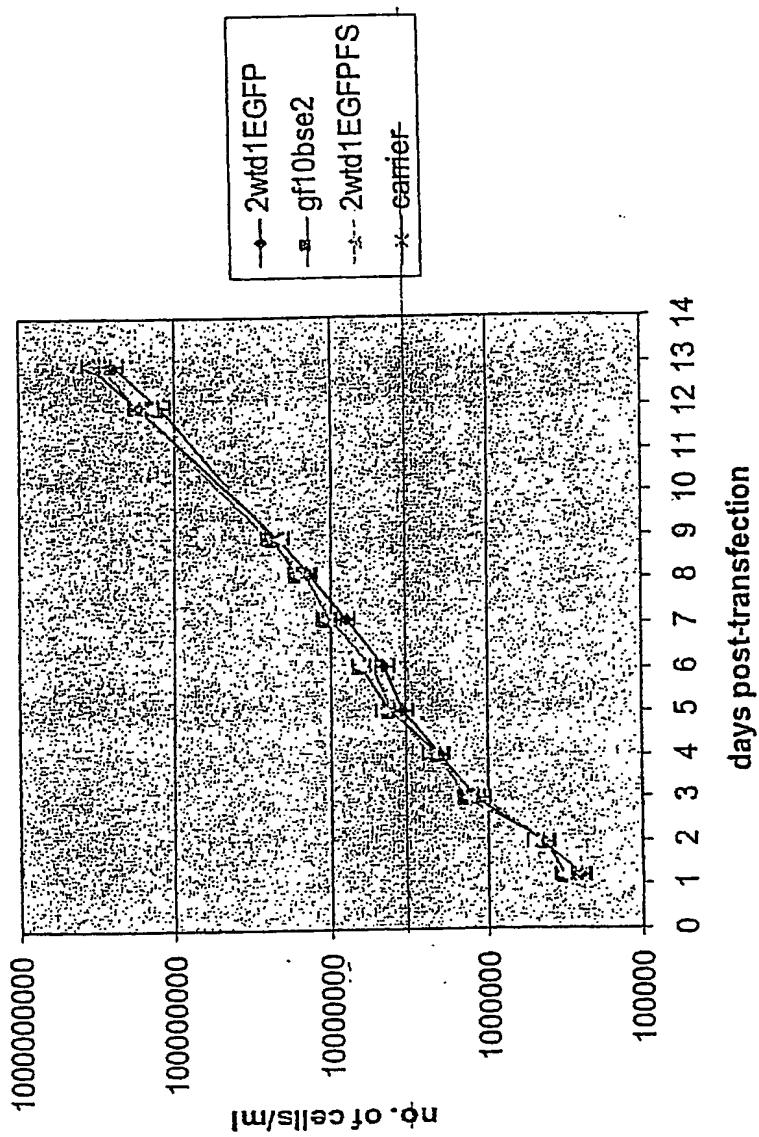


FIG. 21

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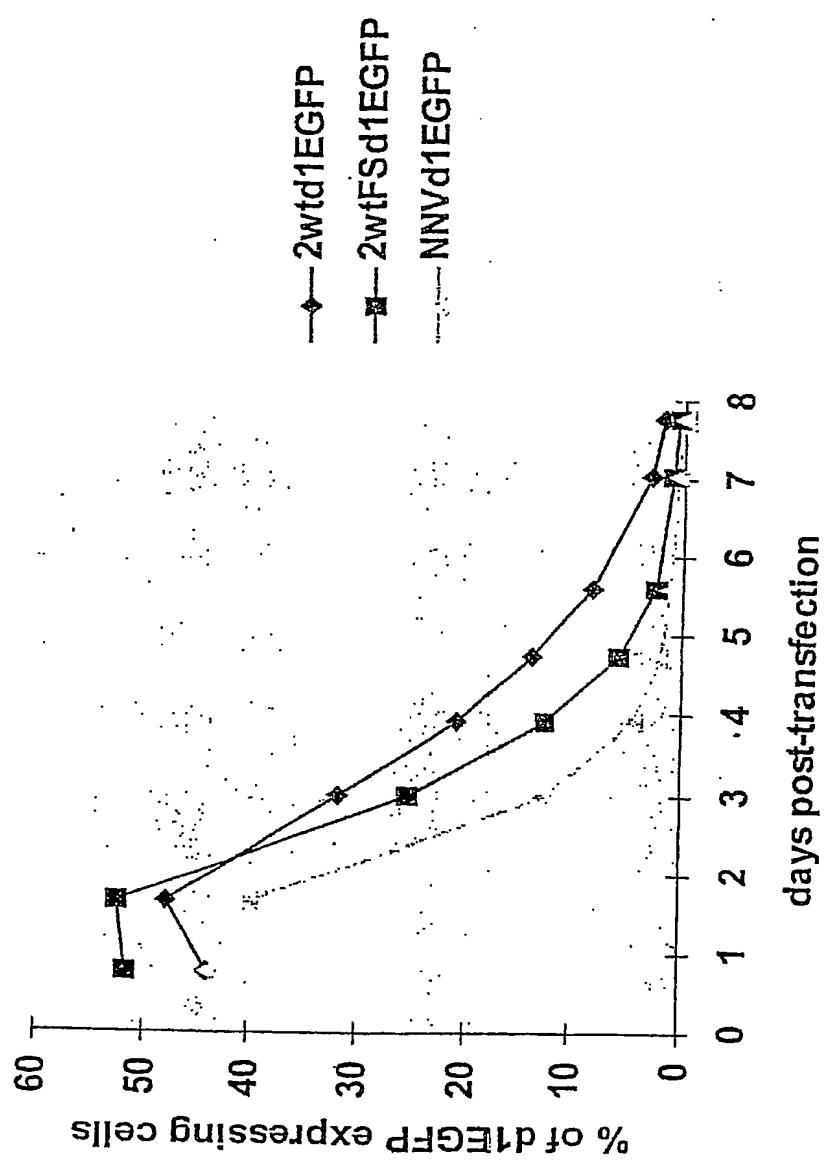


FIG. 22

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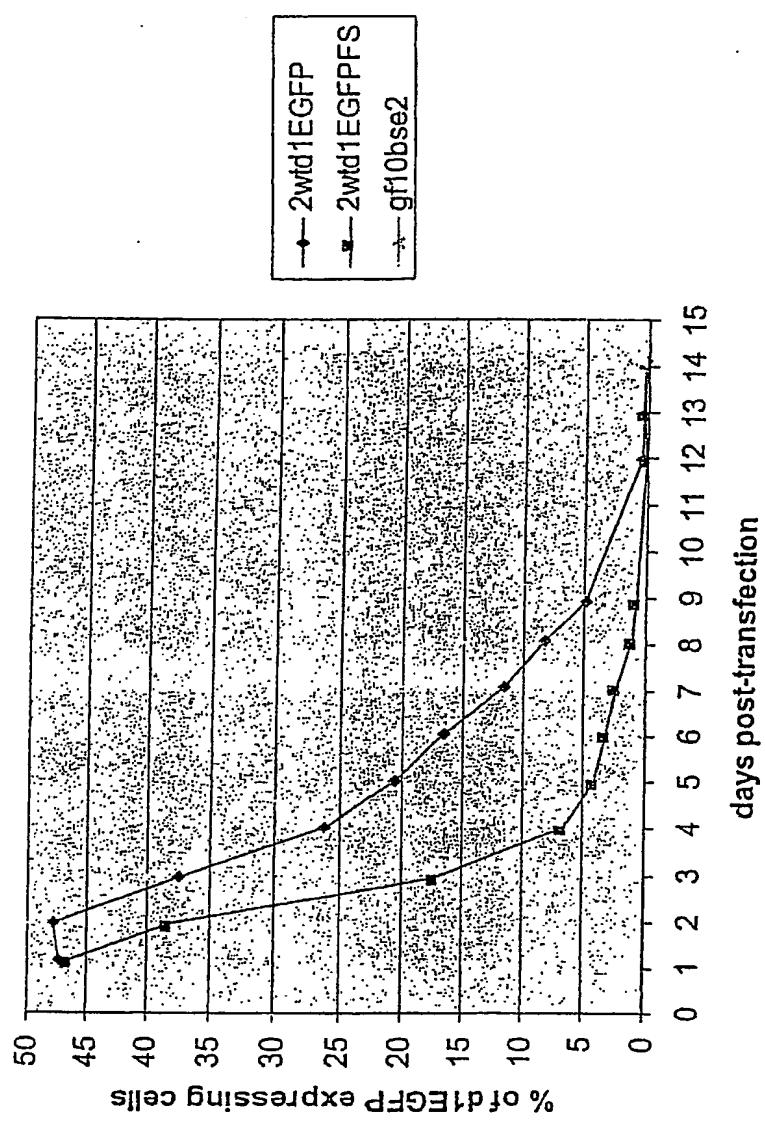


FIG. 23

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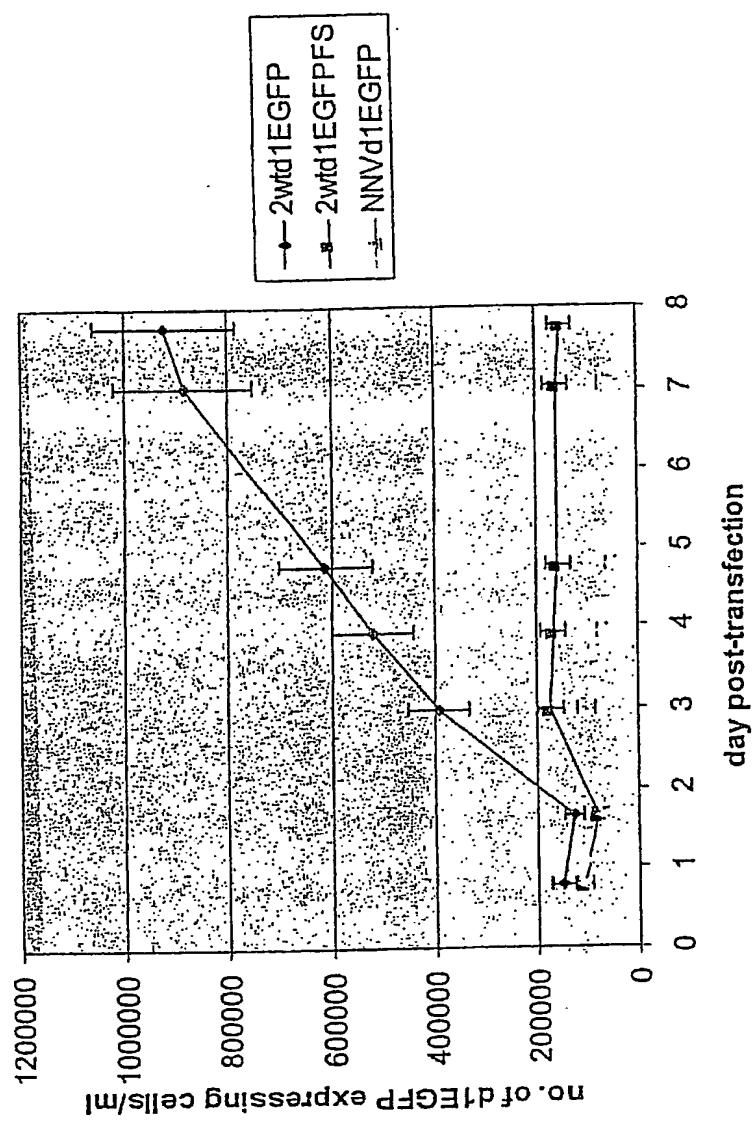


FIG. 24

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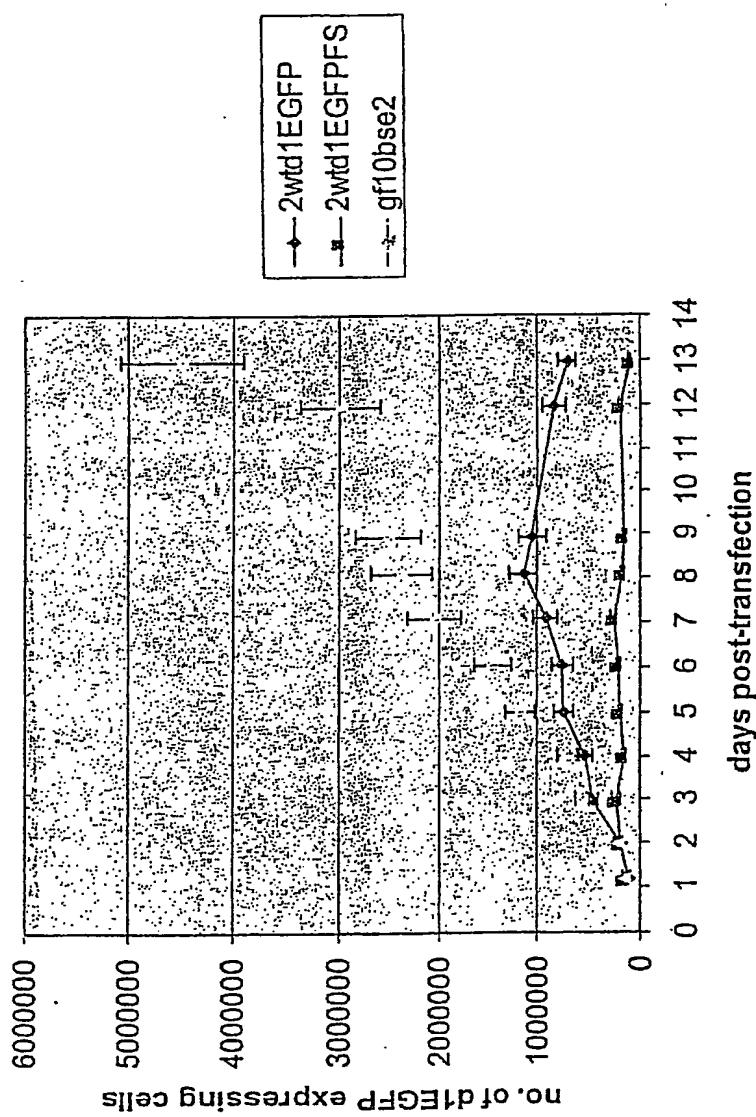


FIG. 25

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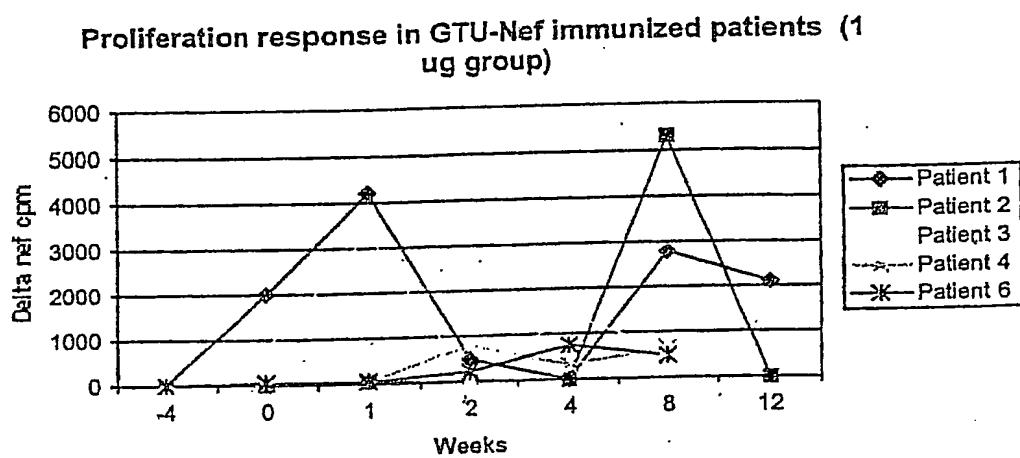
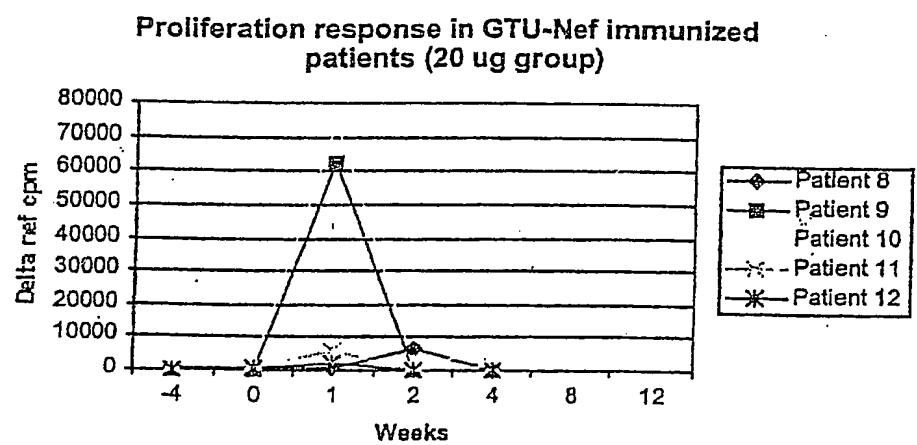


FIG. 26

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**FIG. 27**

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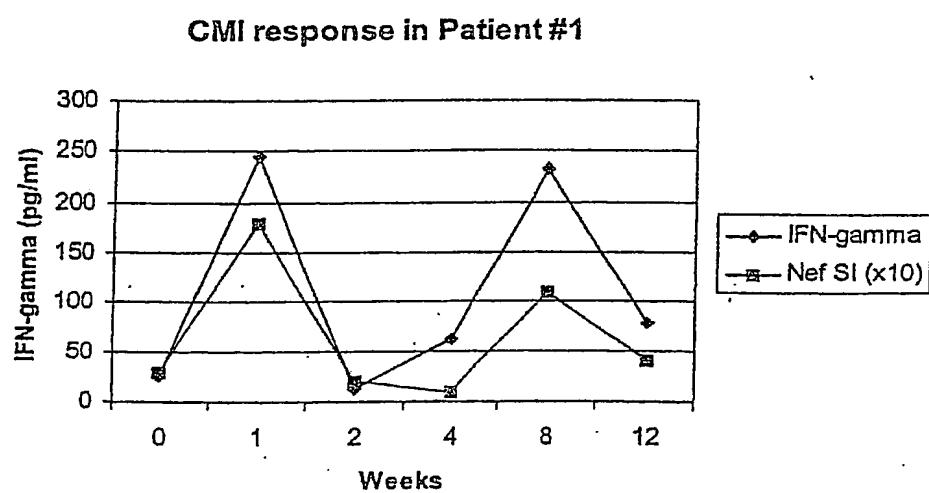


FIG. 28

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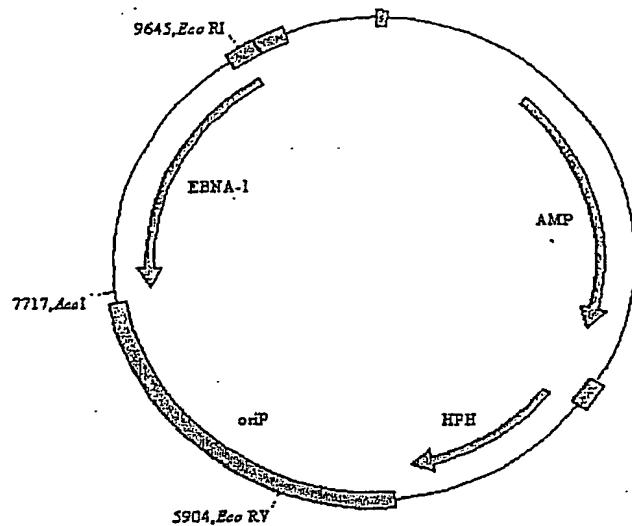
Plasmid pEBO LPP

FIG. 29A

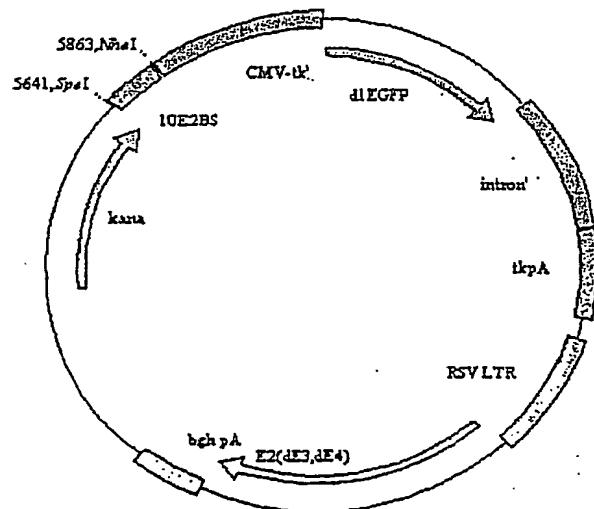
Plasmid s6E2d1EGFP

FIG. 29B

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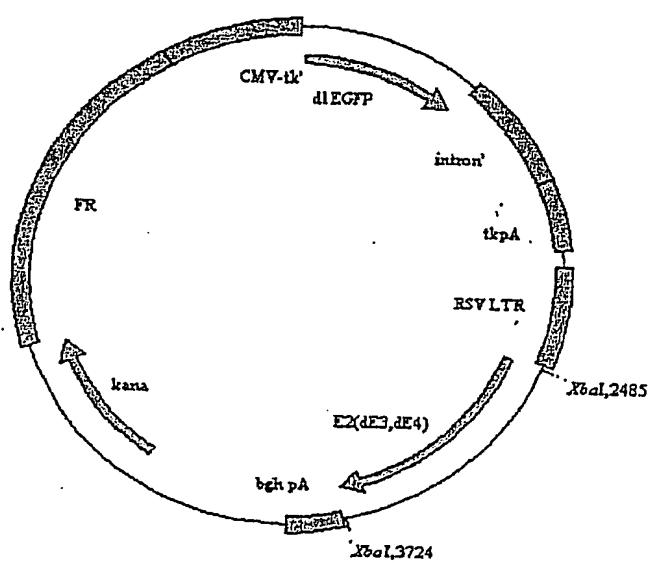
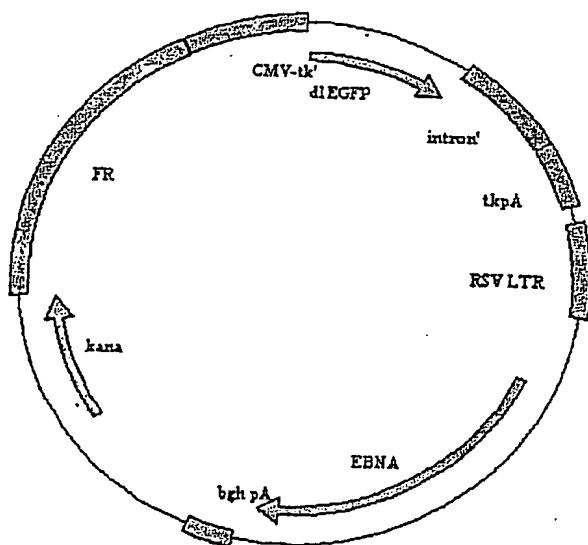
Plasmid FRE2d1EGFP

FIG. 29C

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Plasmid FREBNAd1EGFP**FIG. 30**

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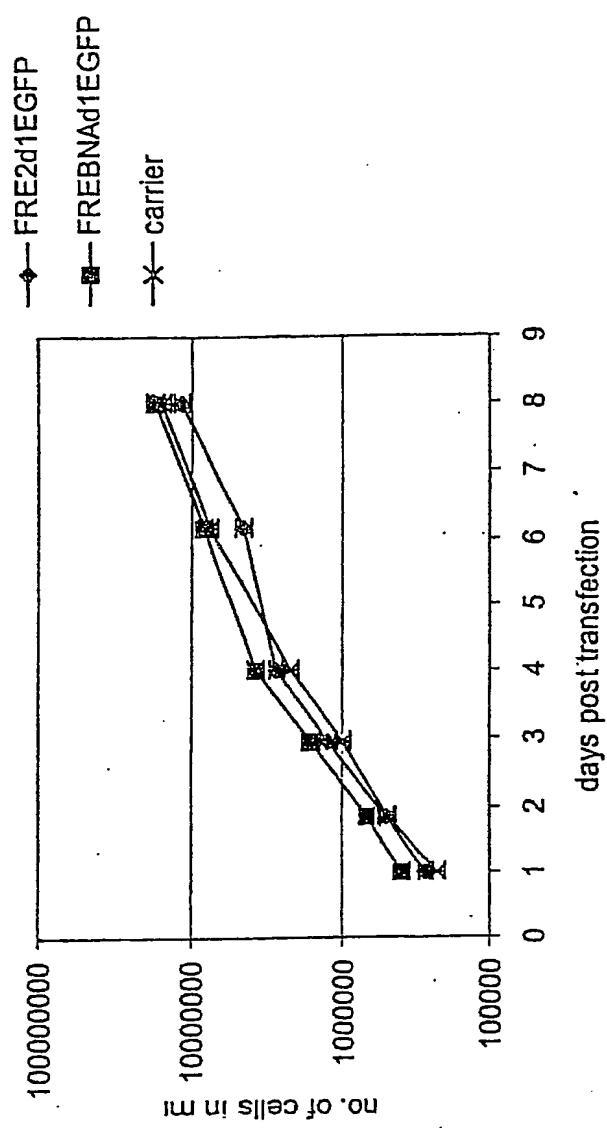


FIG. 31

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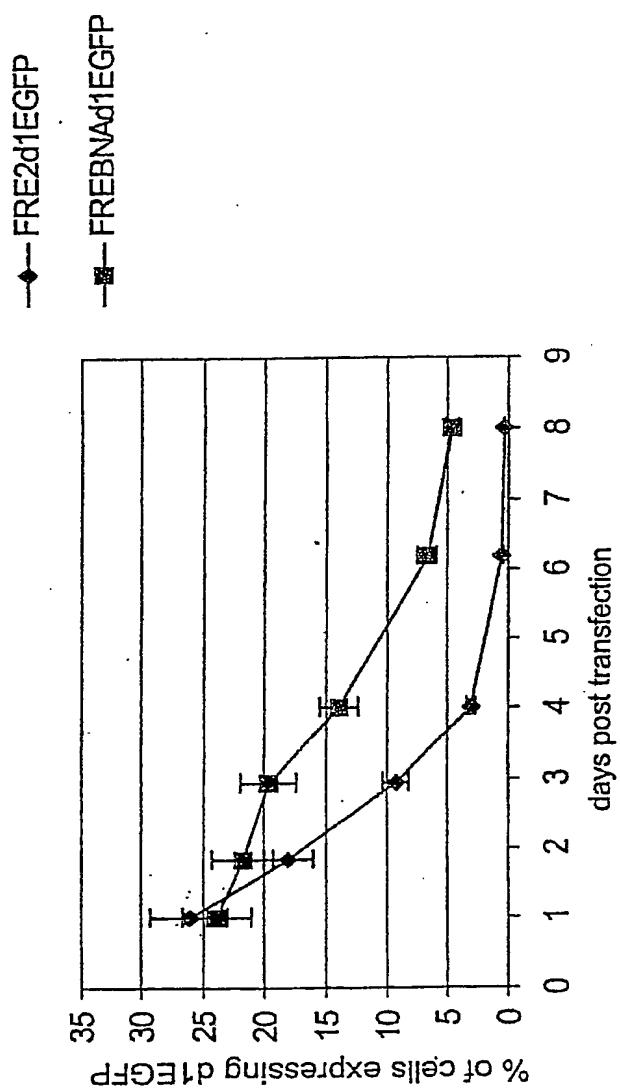


FIG. 32

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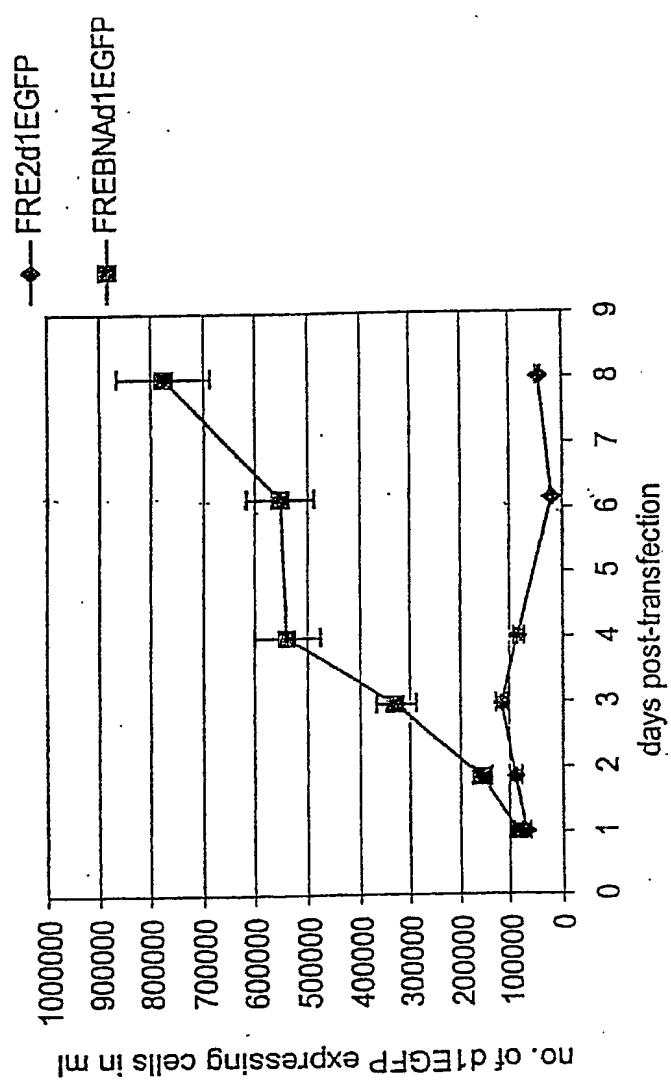


FIG. 33

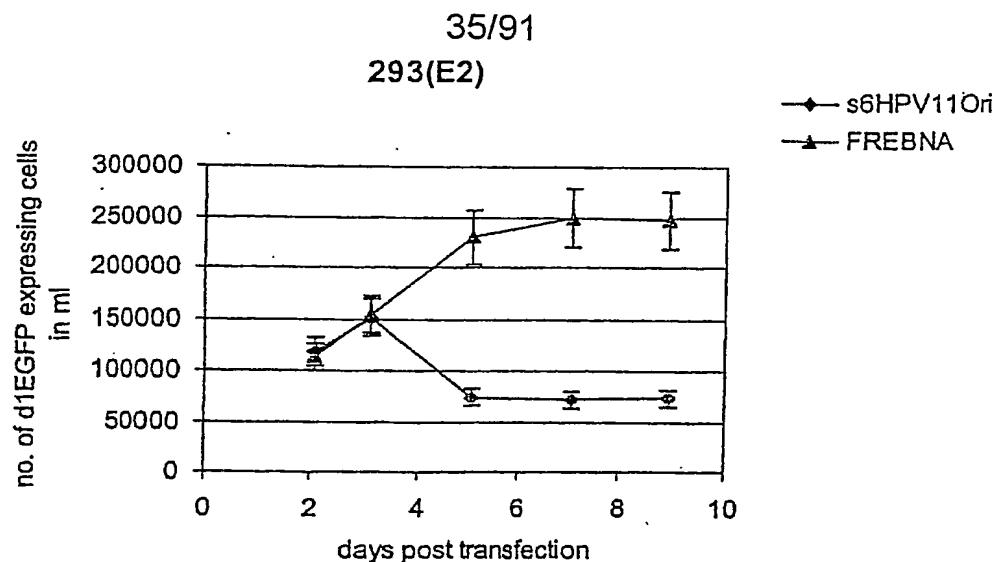


FIG. 34A

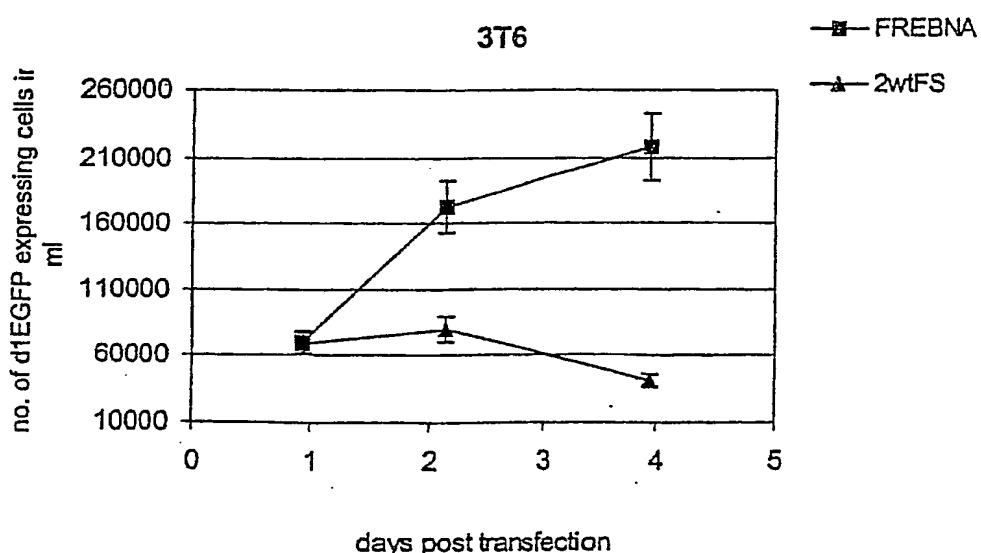
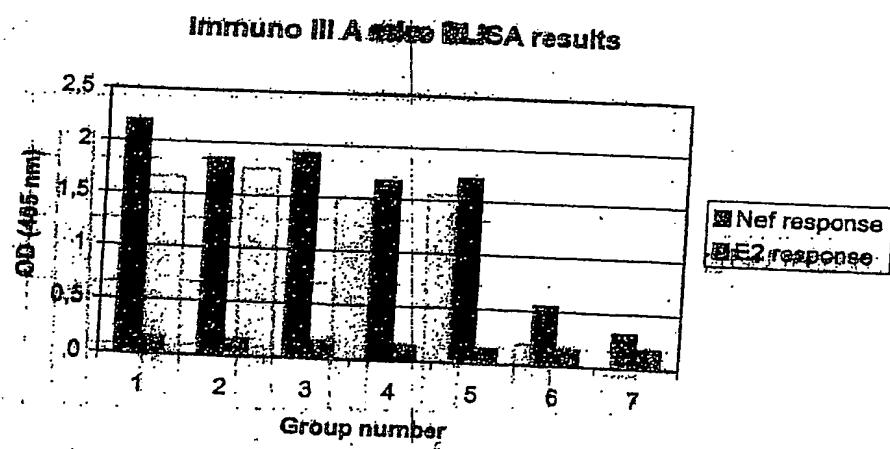


FIG. 34B

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**FIG. 35**

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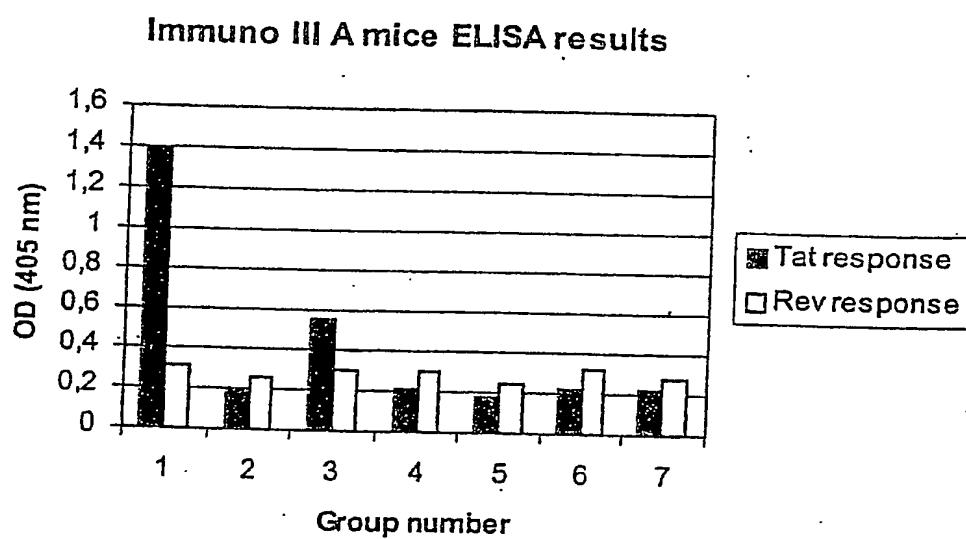
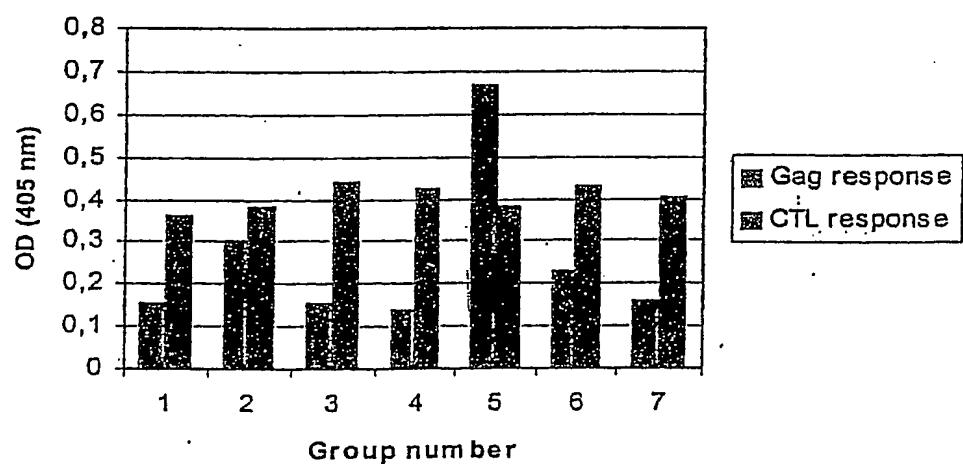
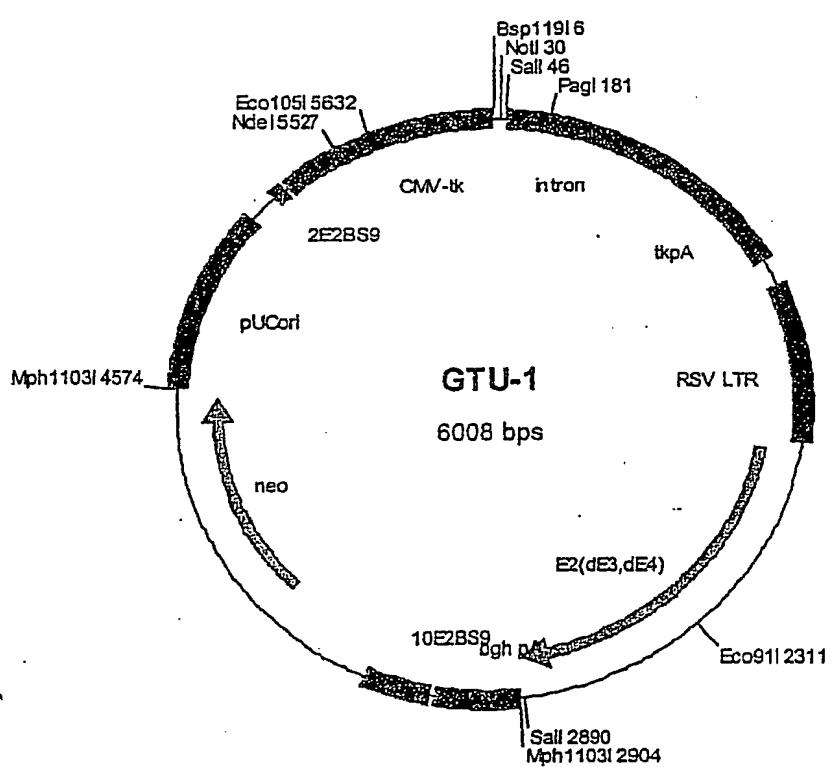


FIG. 36

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Immuno III A mice ELISA results**FIG. 37**

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**FIG. 38A**

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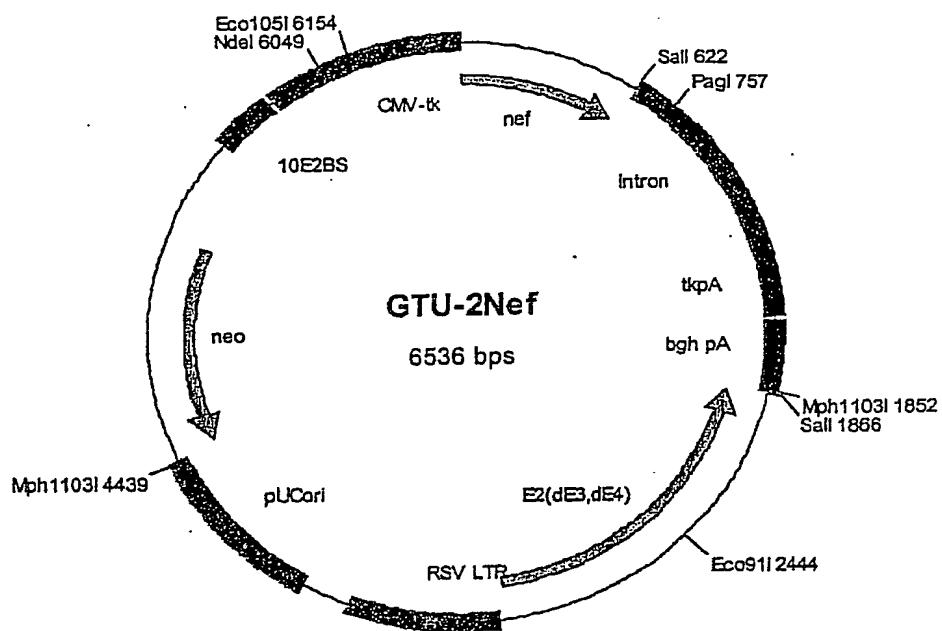


FIG. 38B

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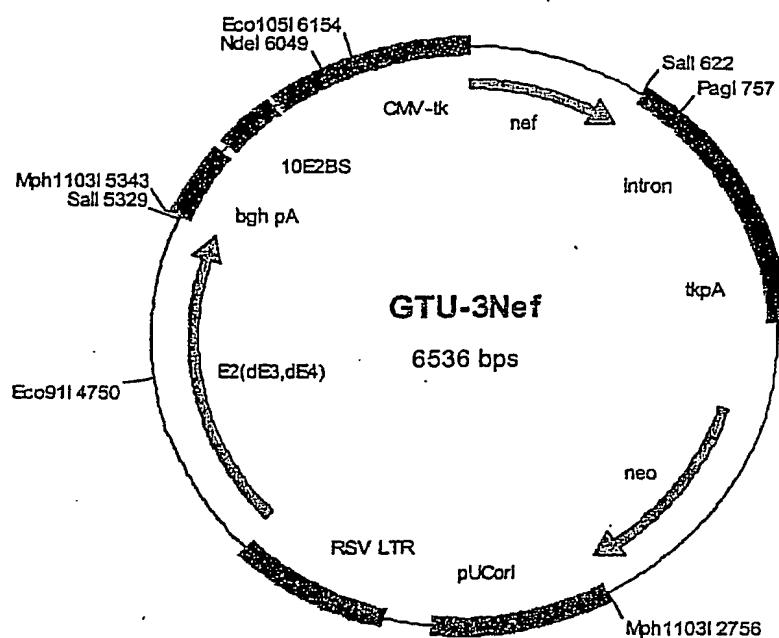
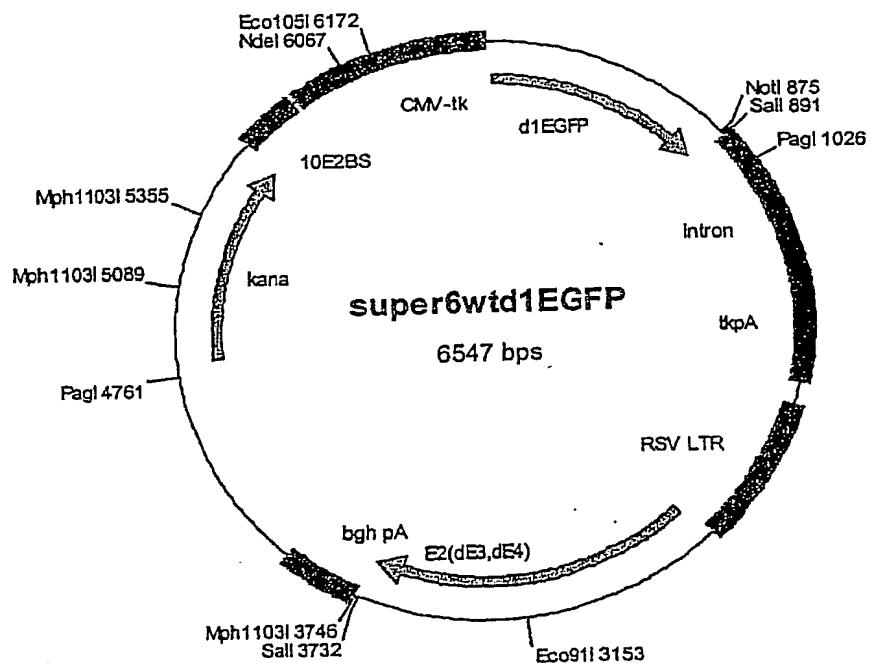


FIG. 38C

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**FIG. 38D**

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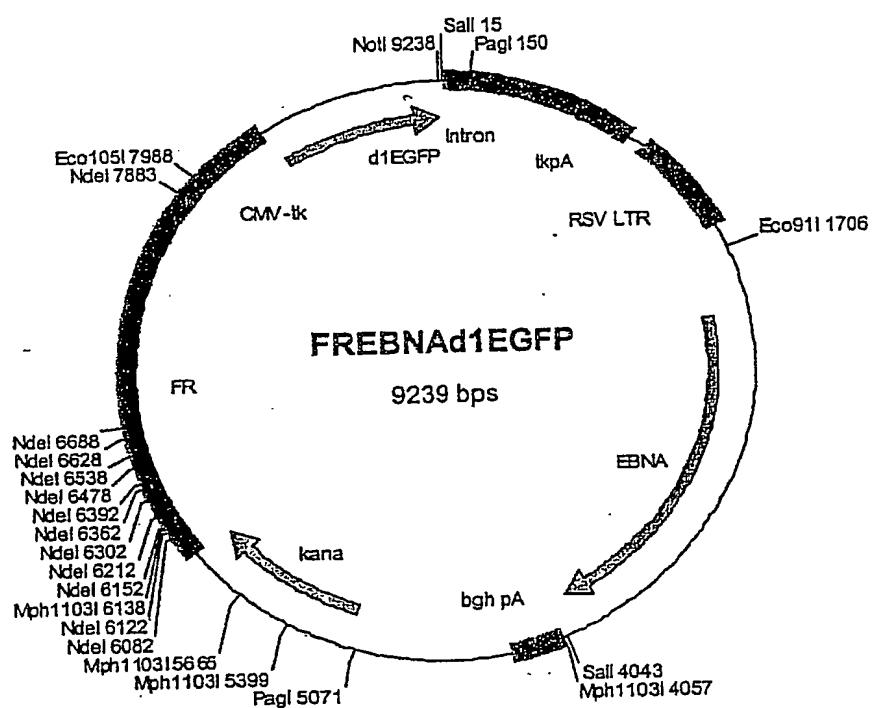


FIG. 38E

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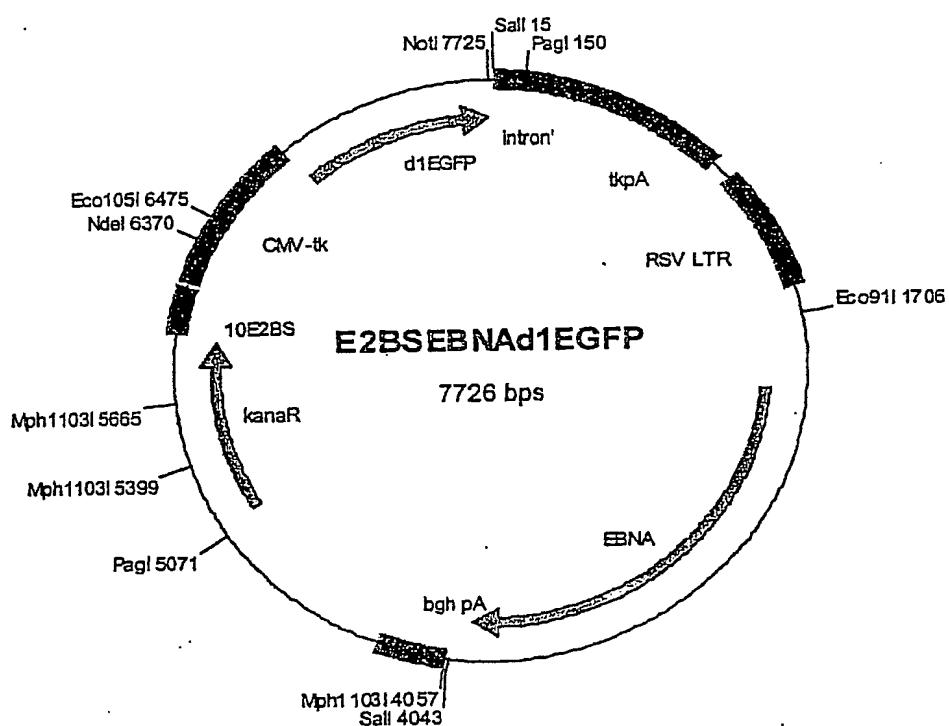


FIG. 38F

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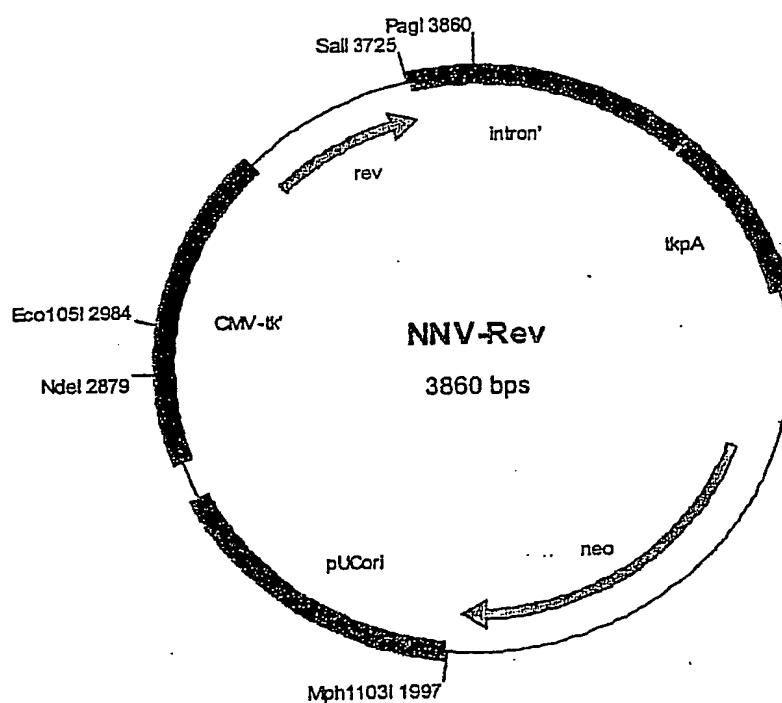


FIG. 38G

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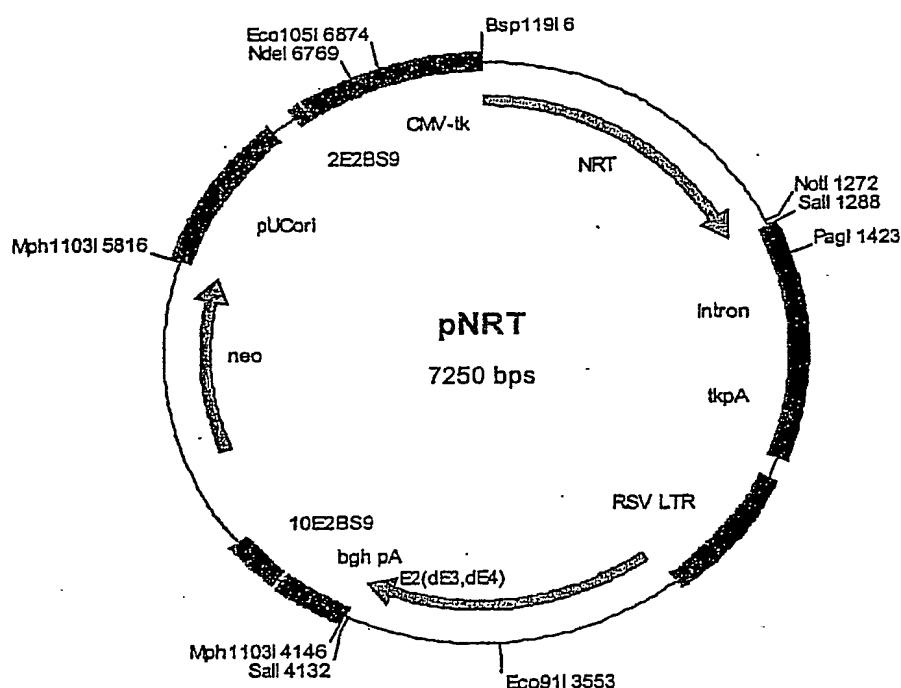


FIG. 39A

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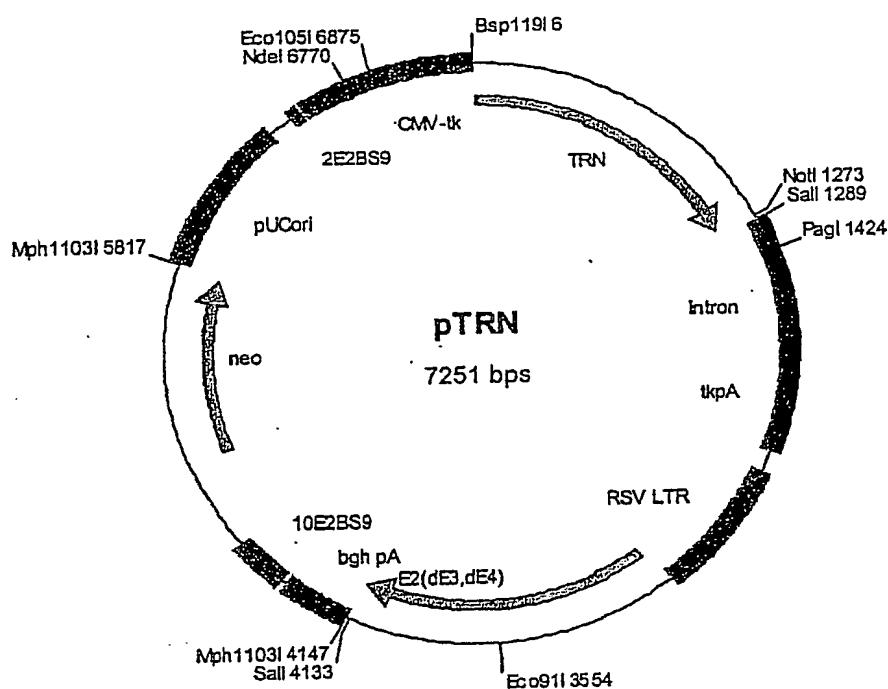


FIG. 39B

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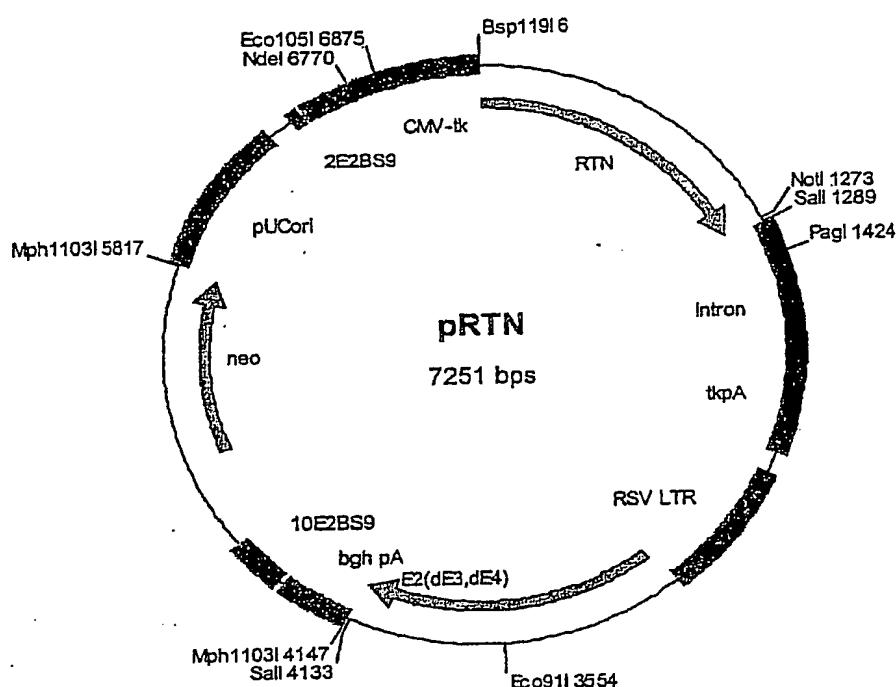


FIG. 39C

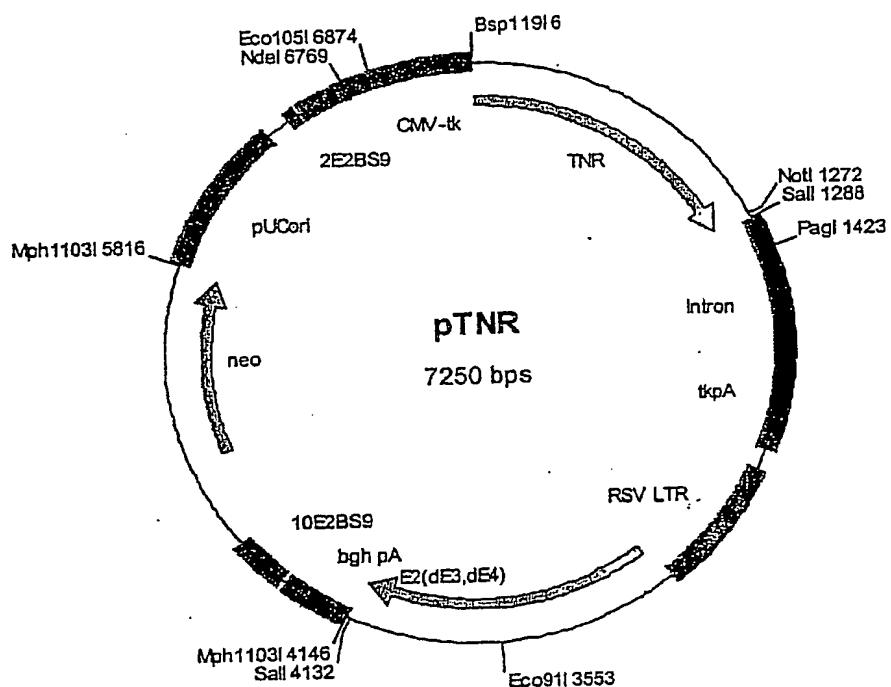


FIG. 39D

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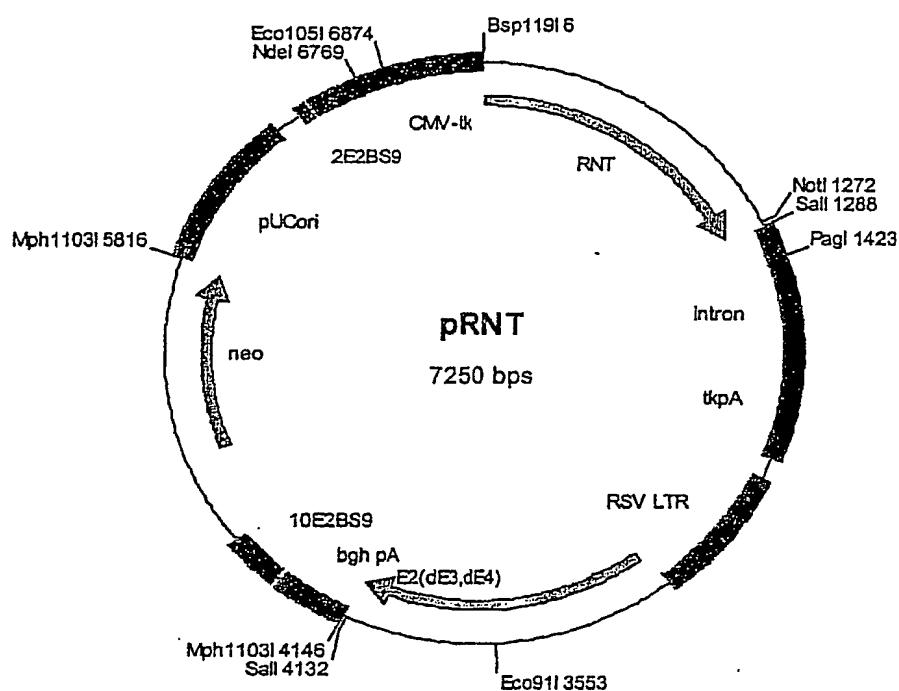


FIG. 39E

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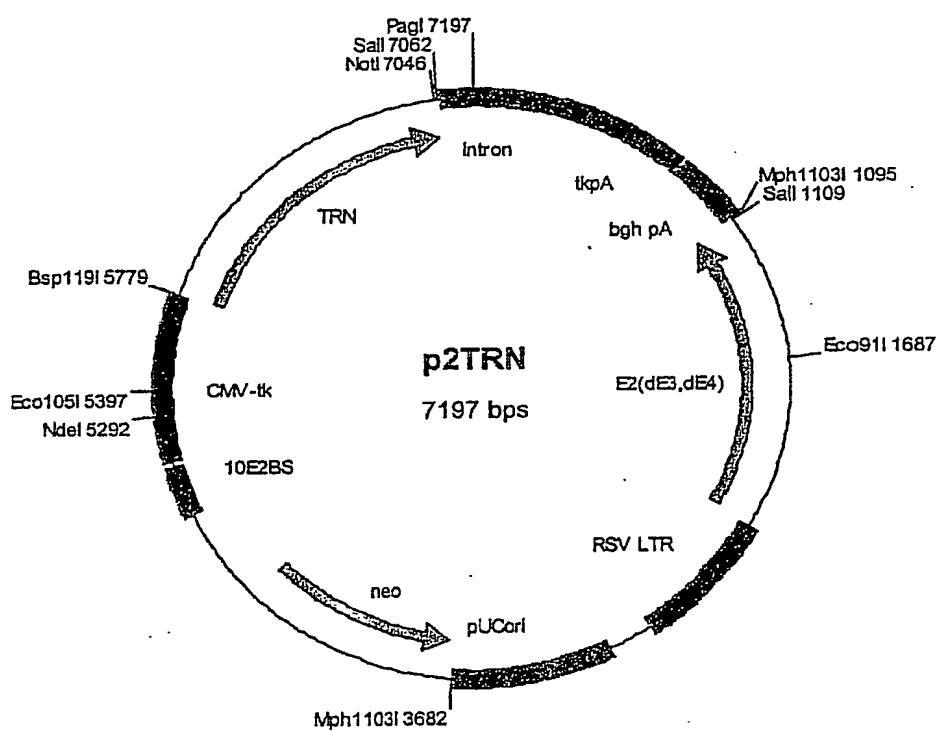


FIG. 39F

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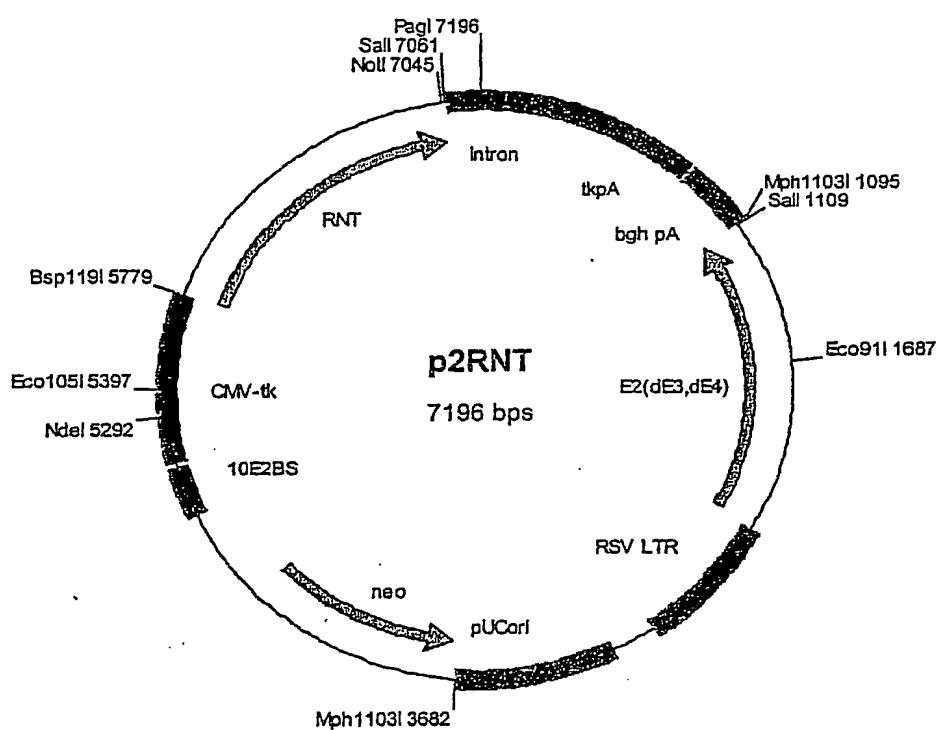


FIG. 39G

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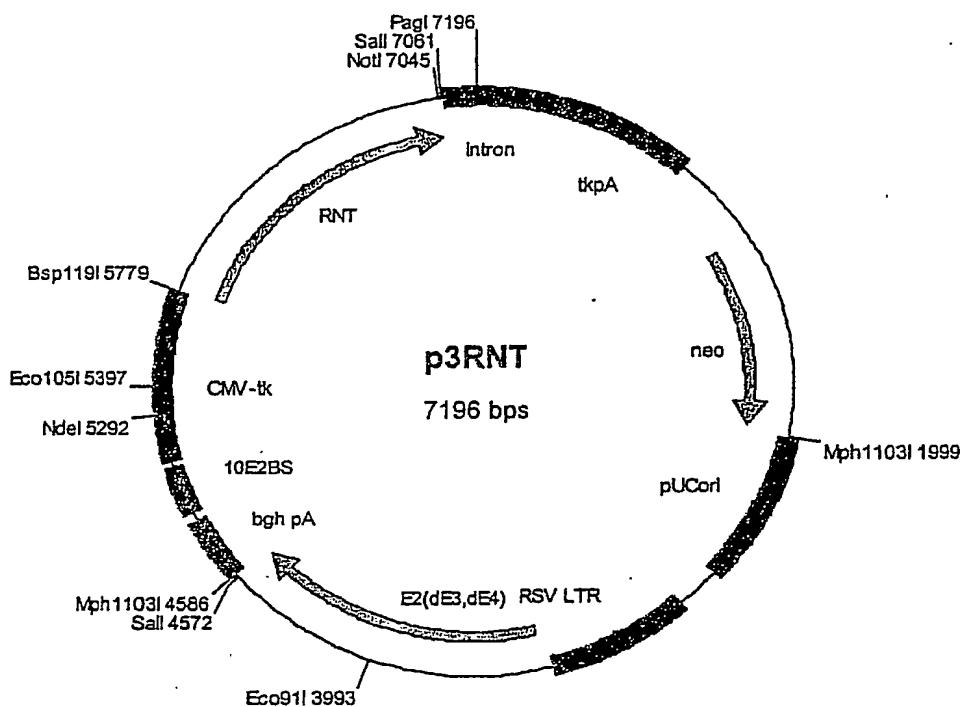


FIG. 39H

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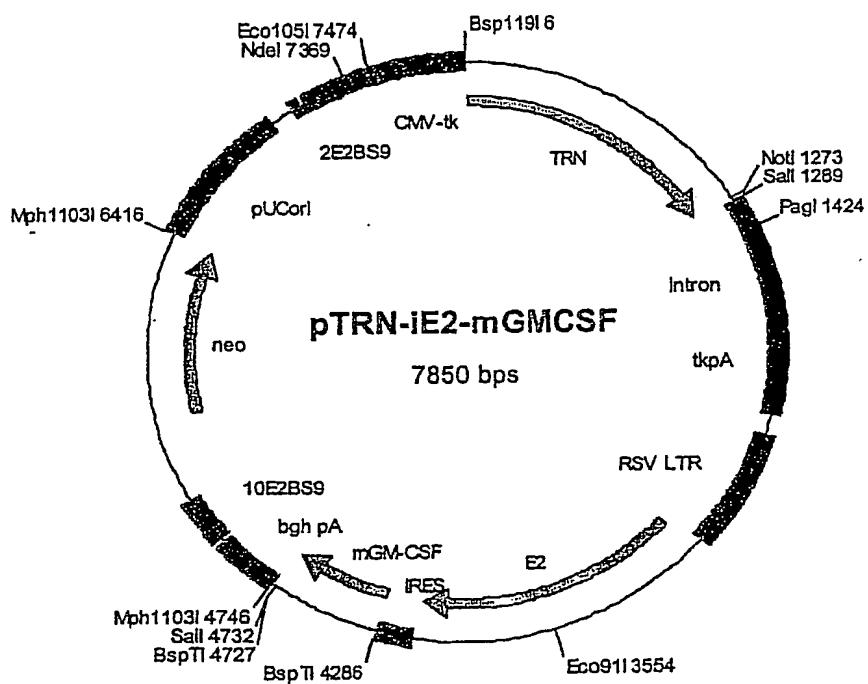


FIG. 39 I

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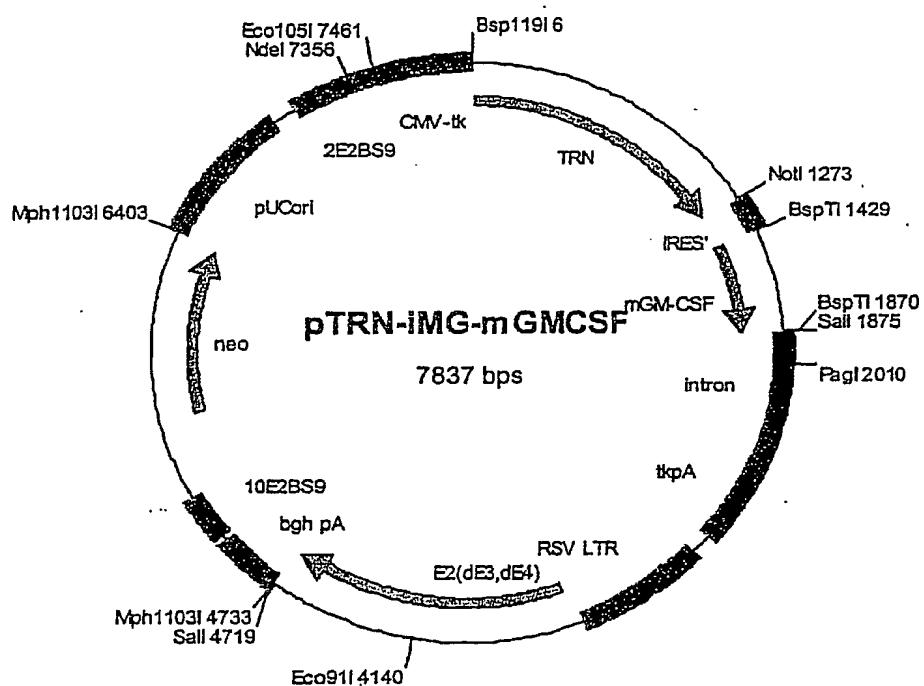


FIG. 39J

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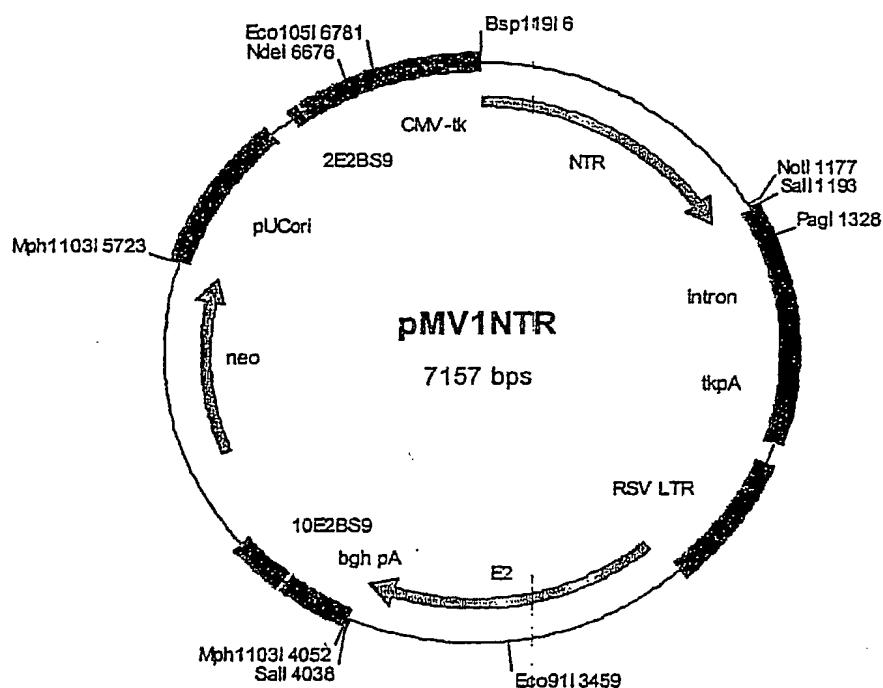


FIG. 40A

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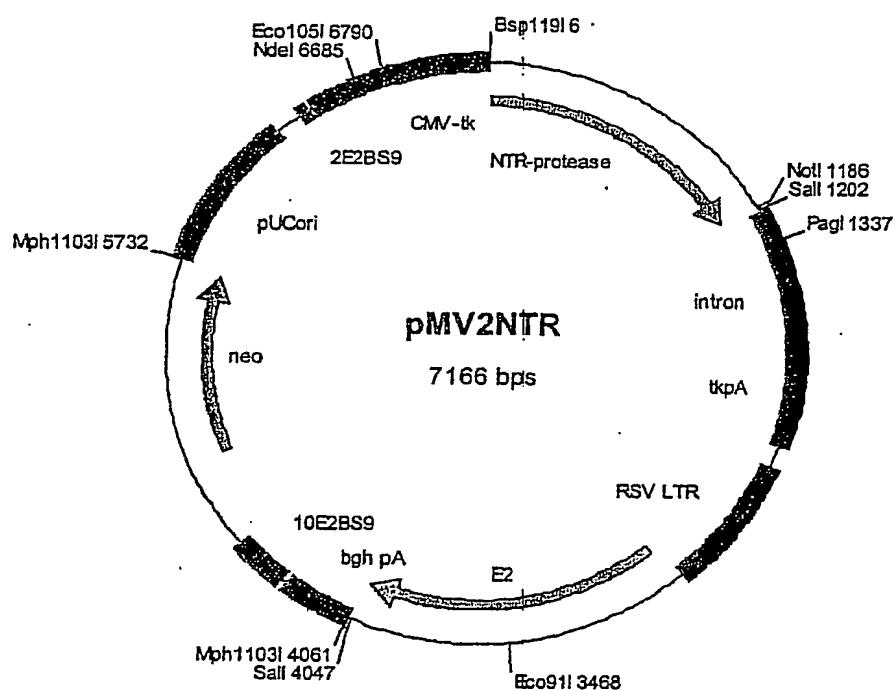


FIG. 40B

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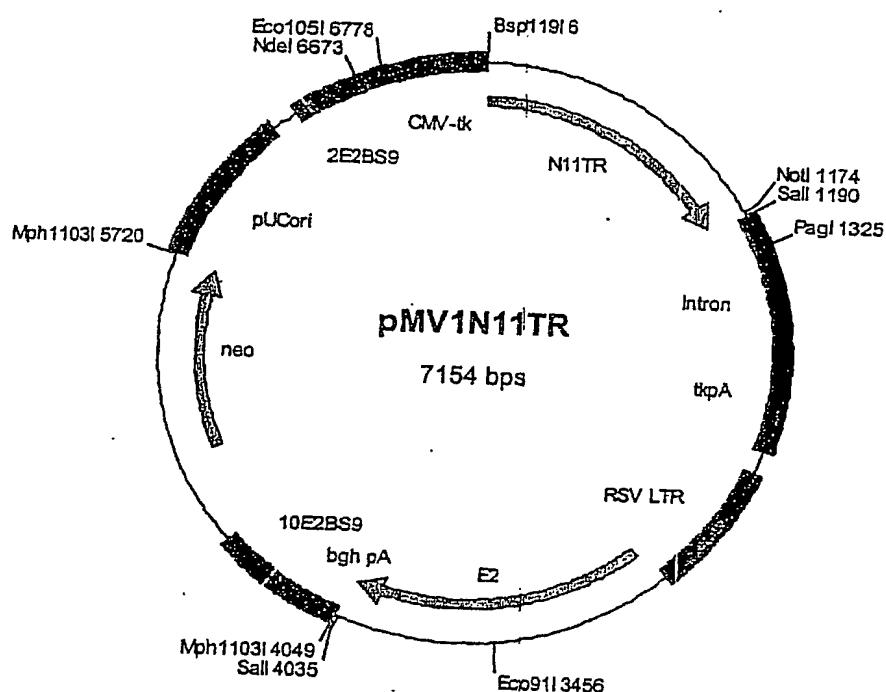


FIG. 40C

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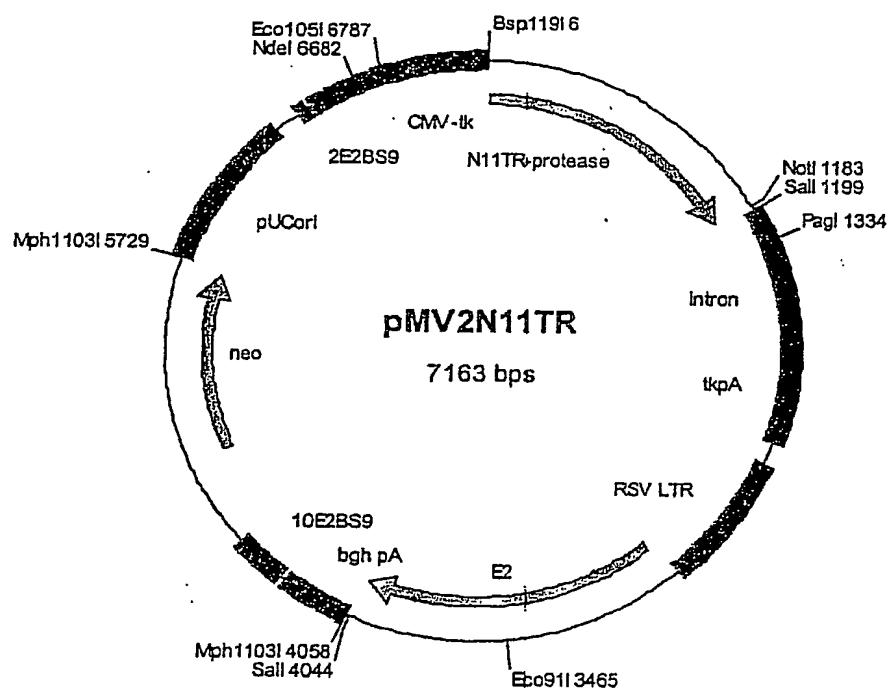


FIG. 40D

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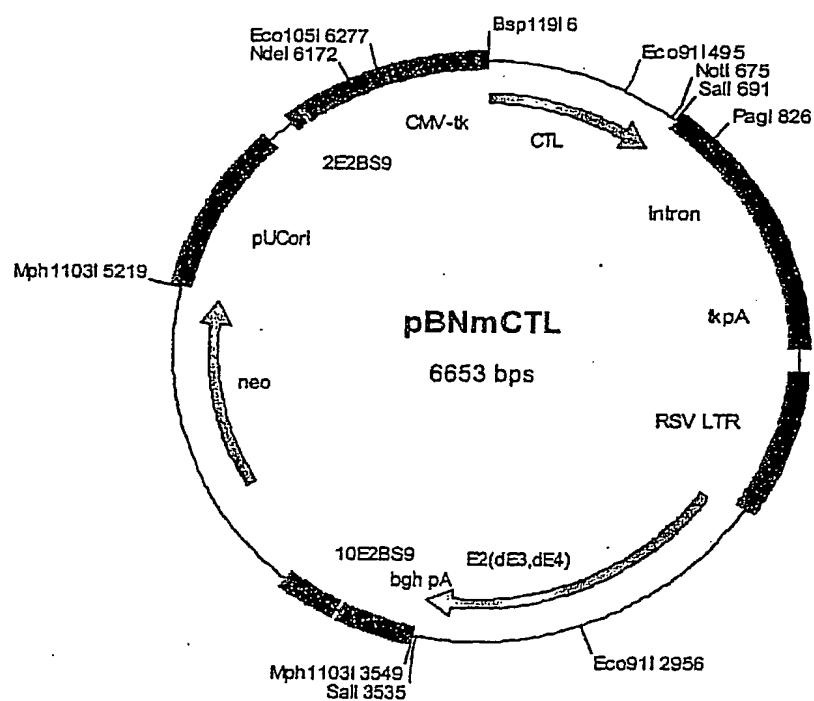


FIG. 41A

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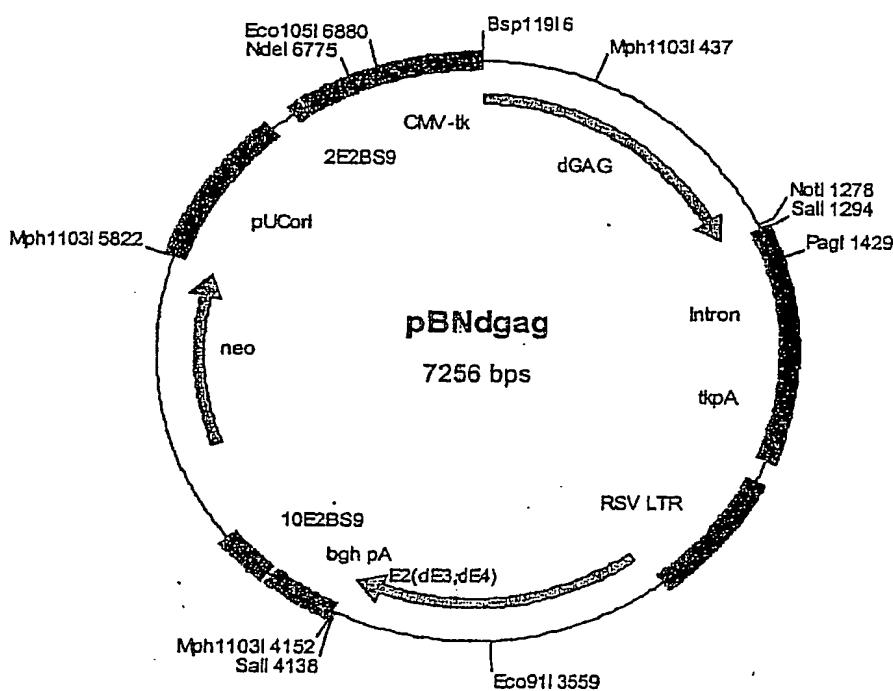


FIG. 41B

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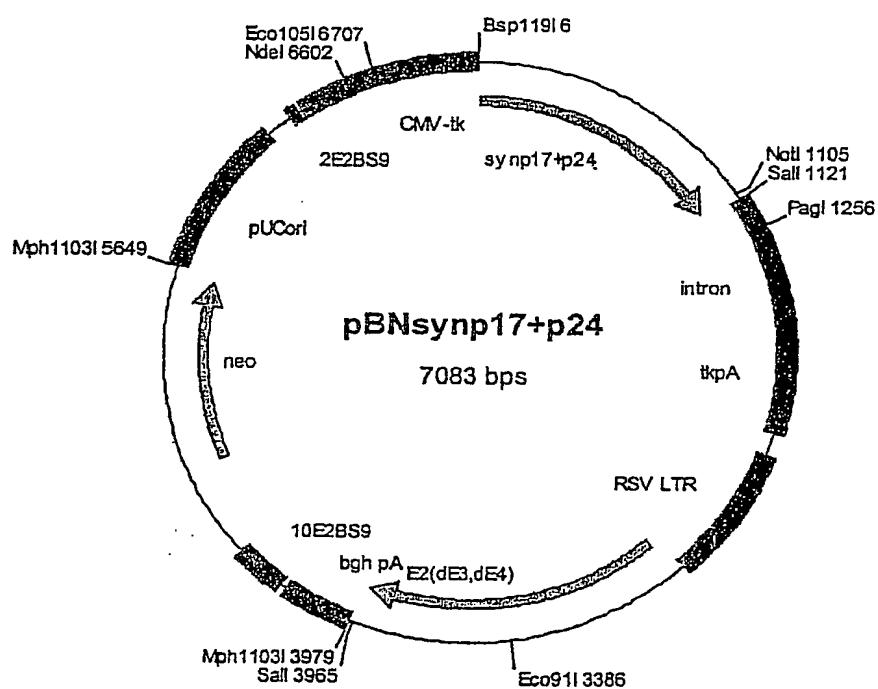


FIG. 41C

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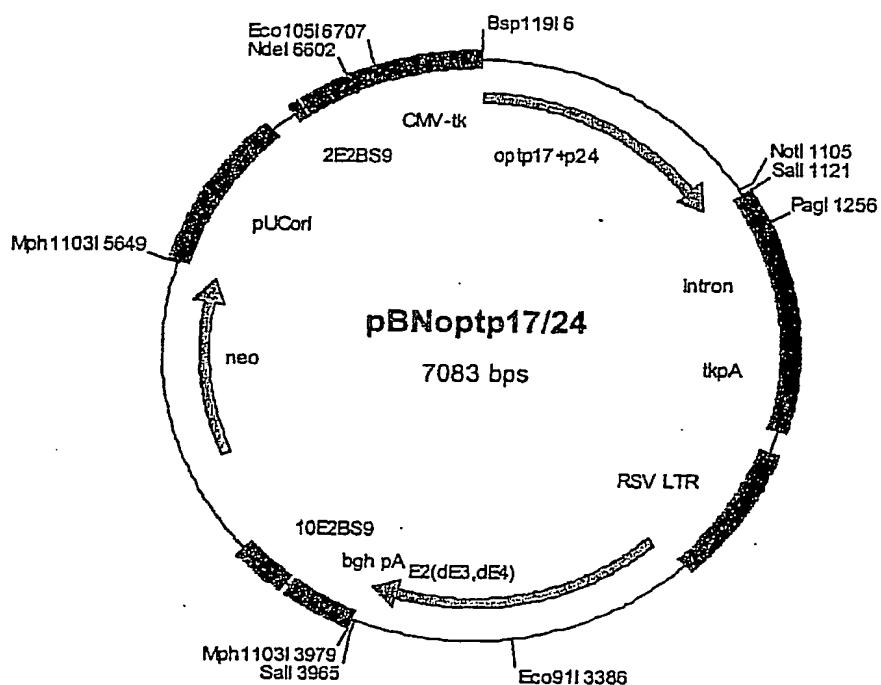


FIG. 41D

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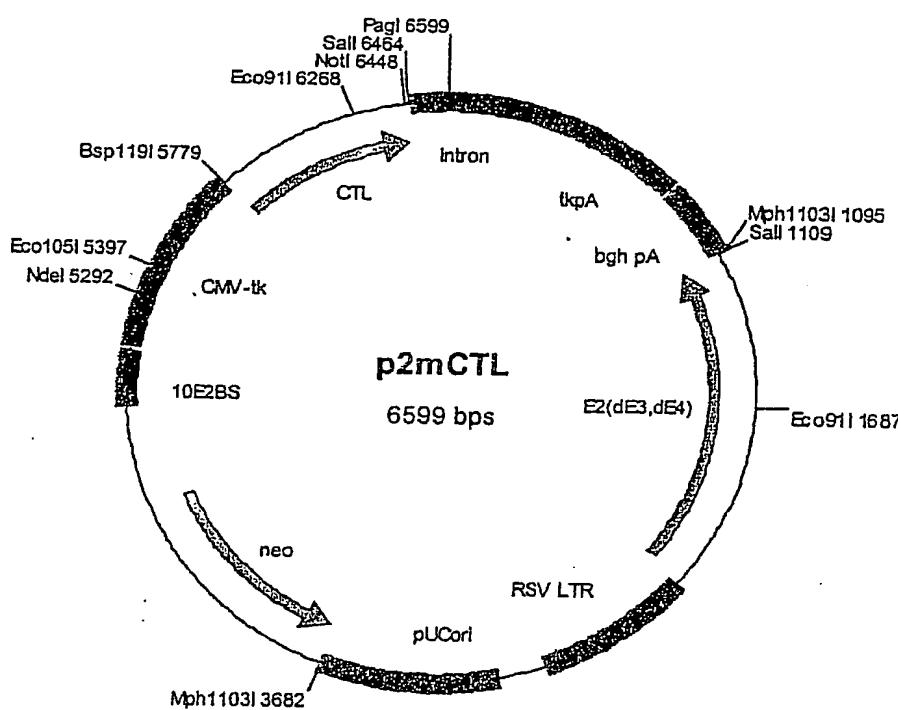


FIG. 41E

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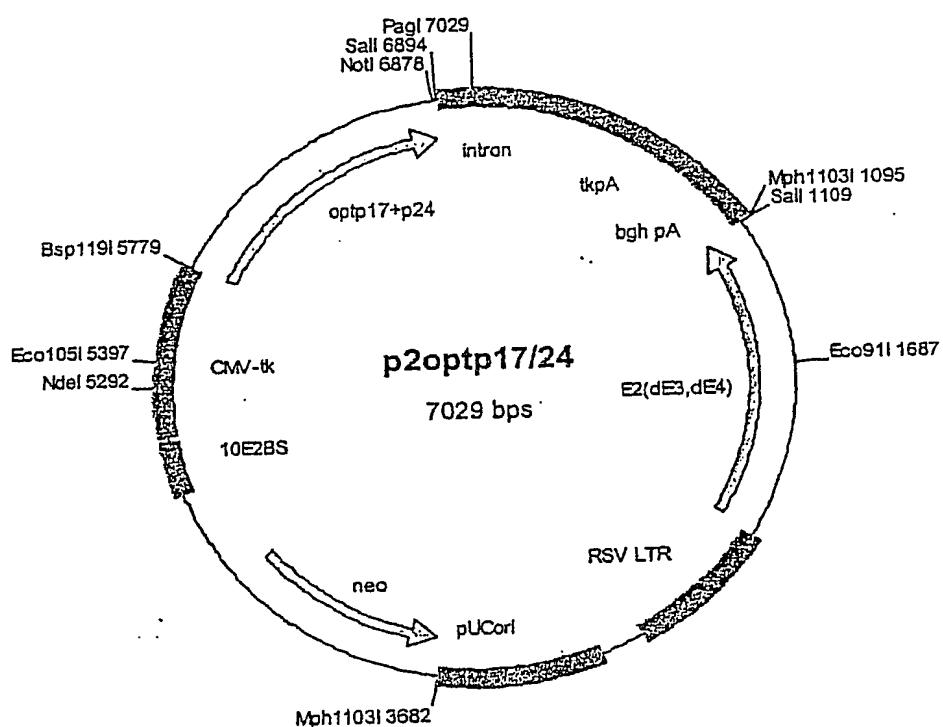


FIG. 41F

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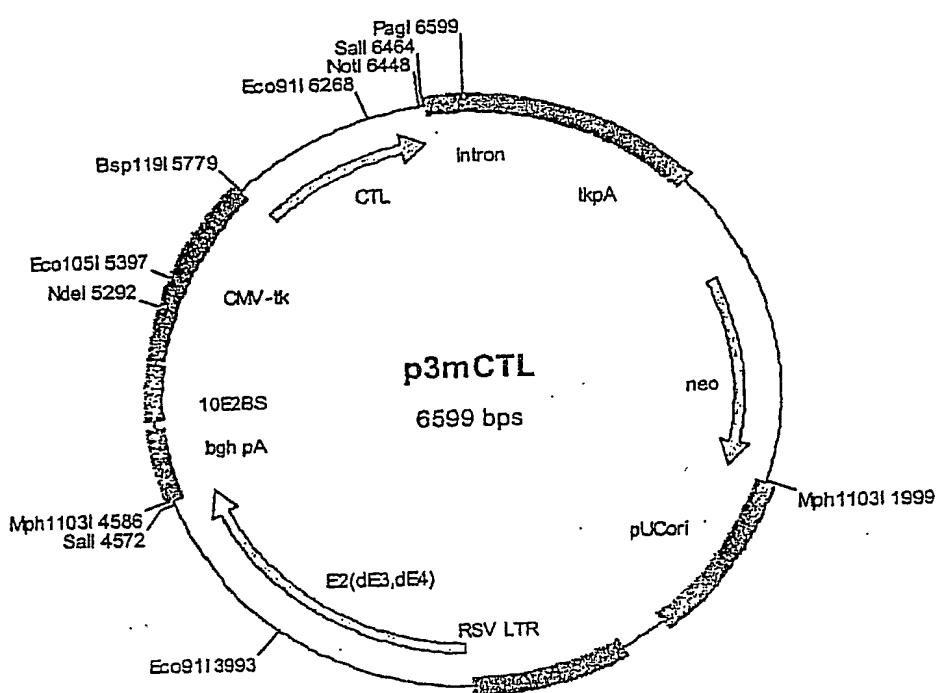


FIG. 41G

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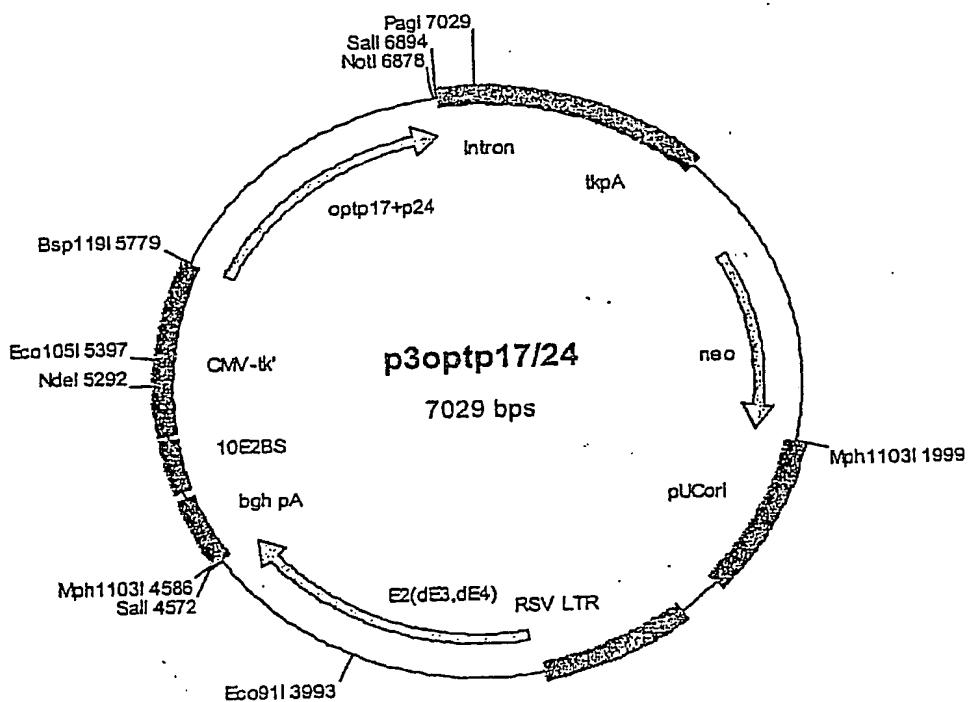


FIG. 41H

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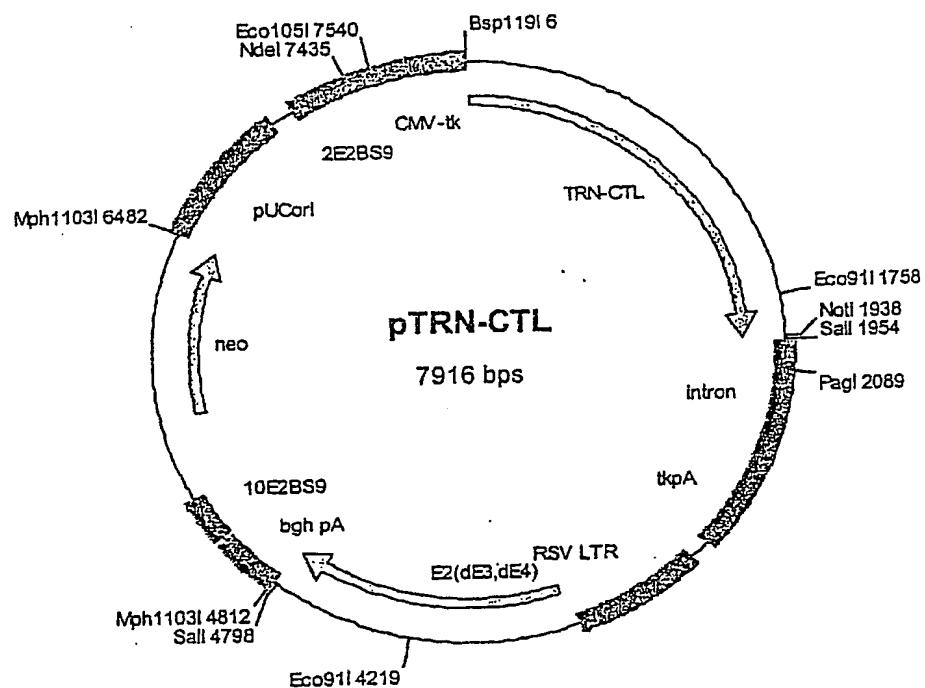


FIG. 42A

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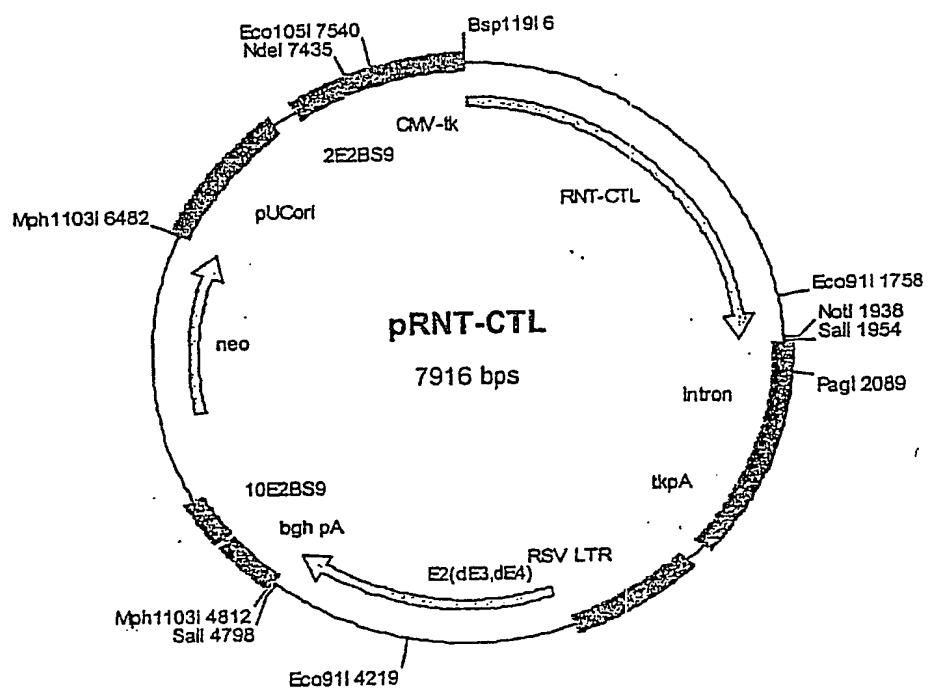


FIG. 42B

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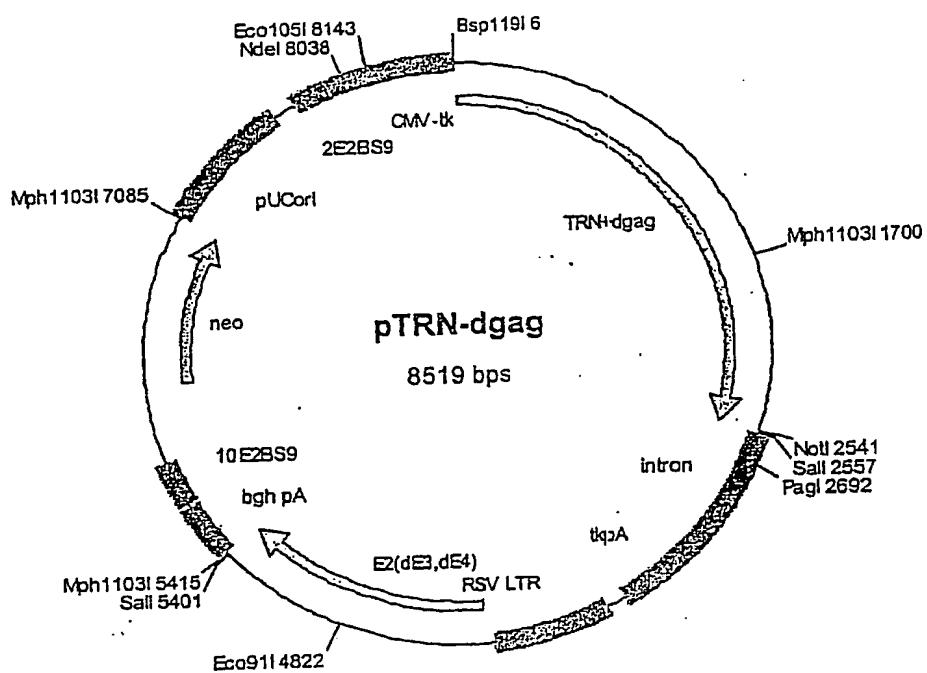


FIG. 42C

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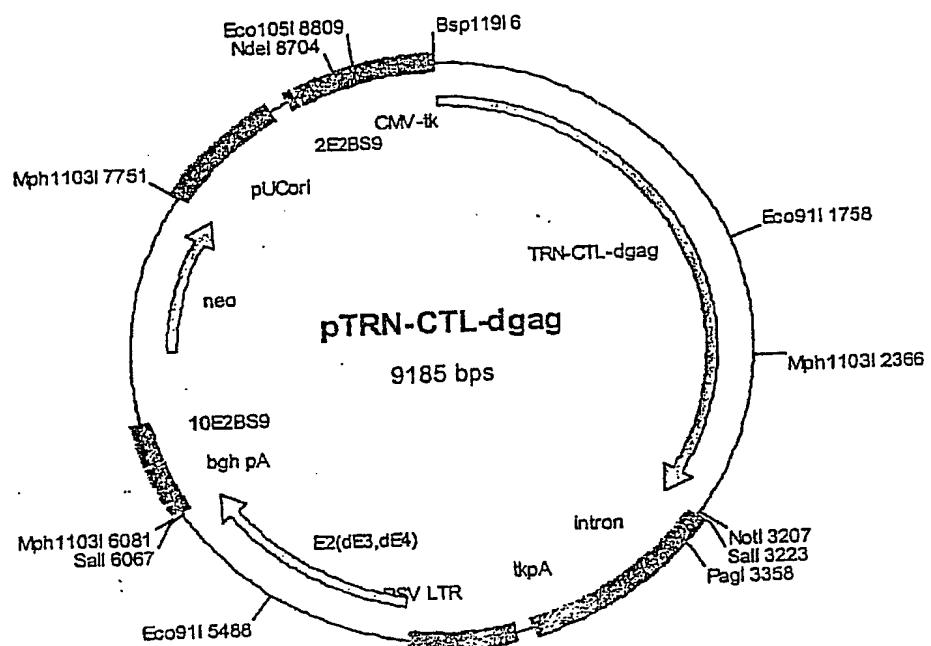


FIG. 42D

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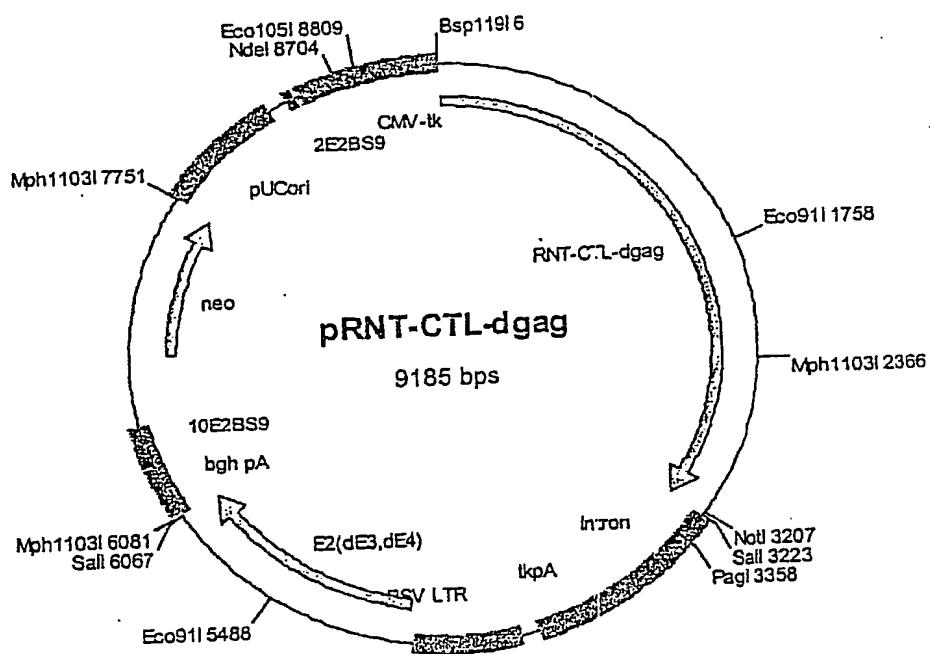


FIG. 42E

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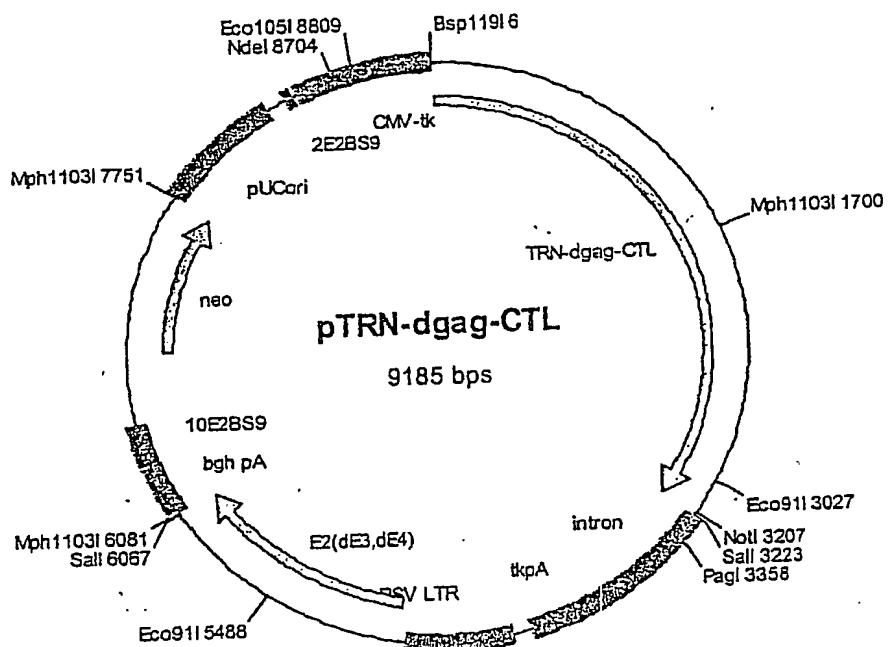


FIG. 42F

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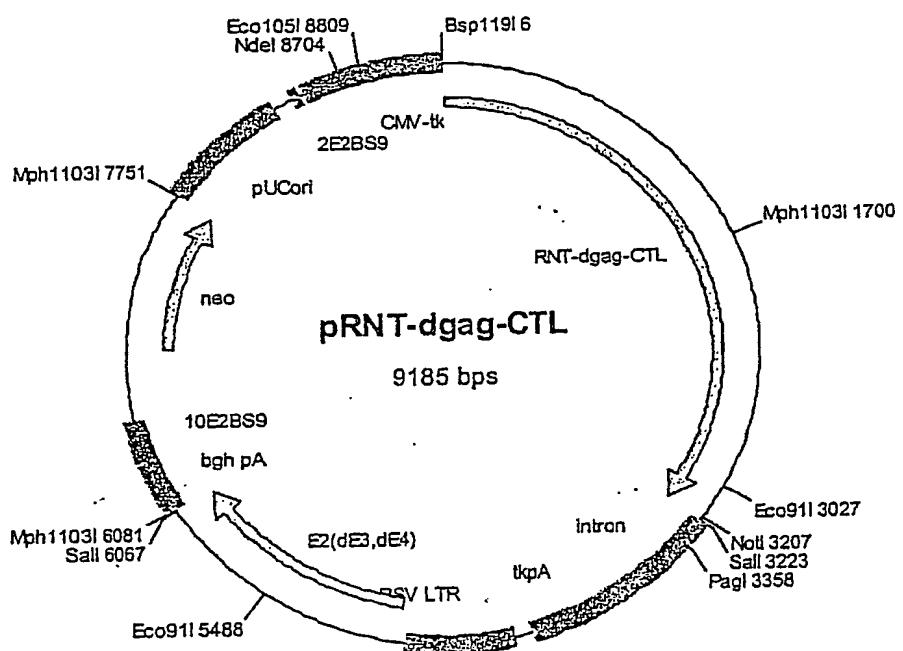


FIG. 42G

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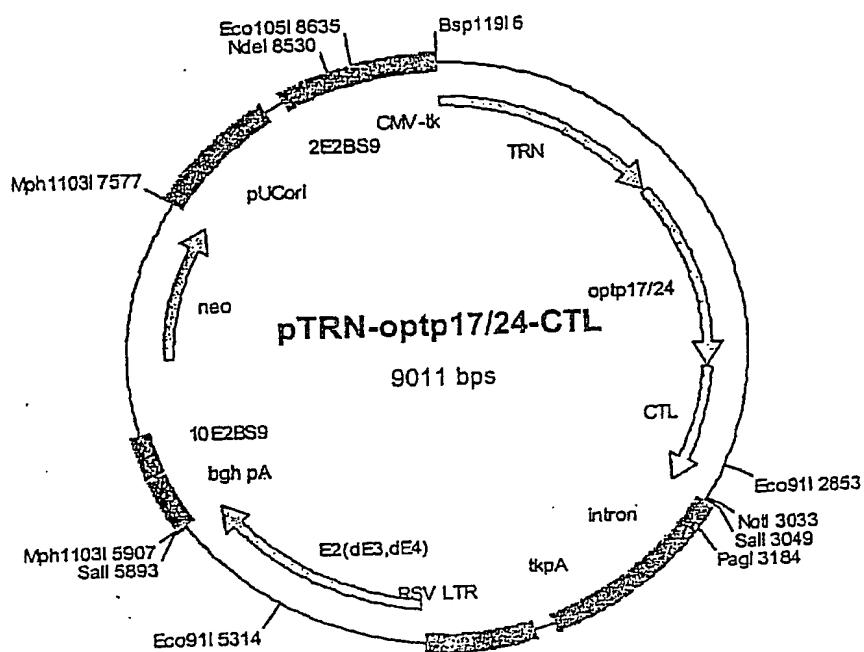


FIG. 42H

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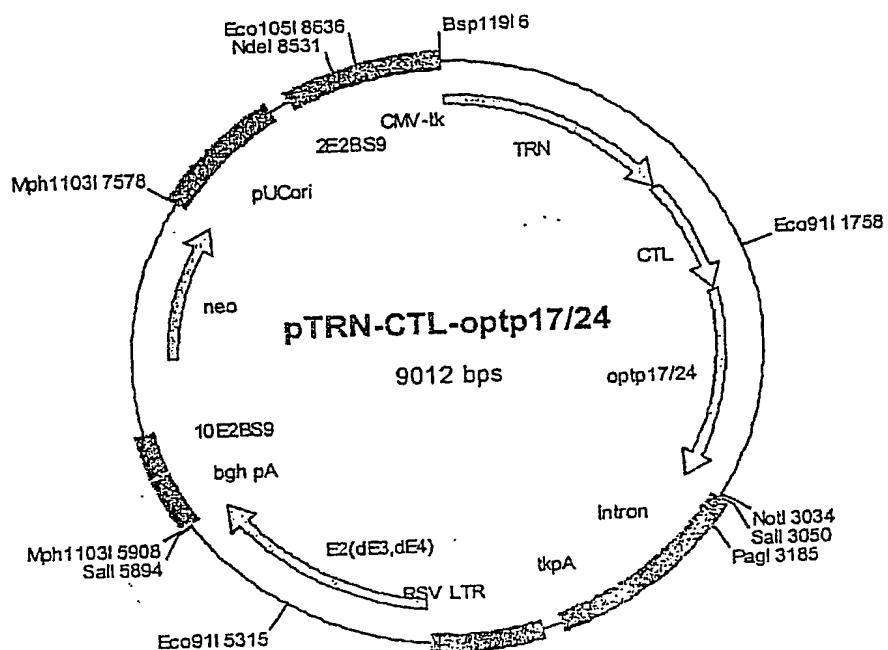


FIG. 42 I

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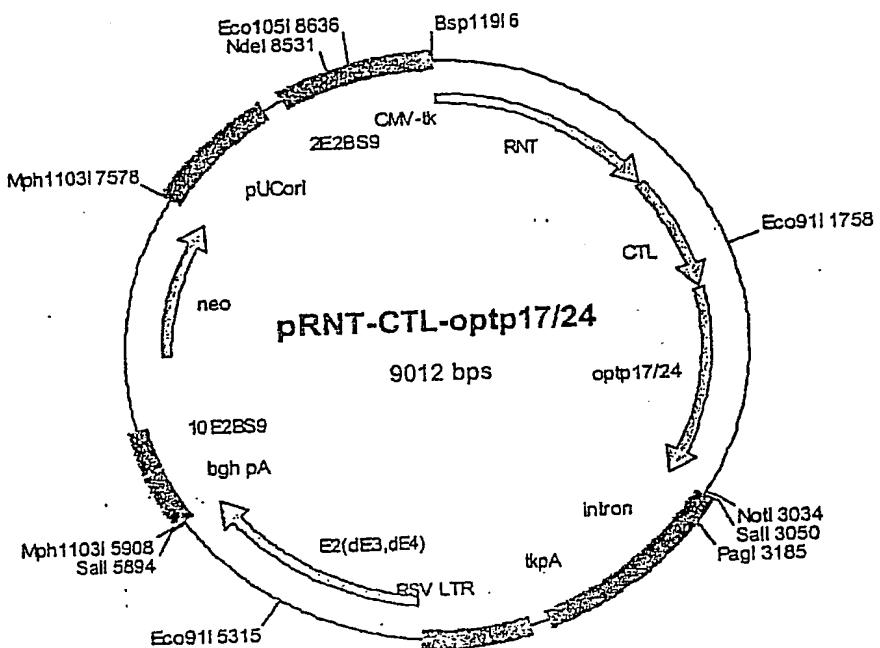


FIG. 42J

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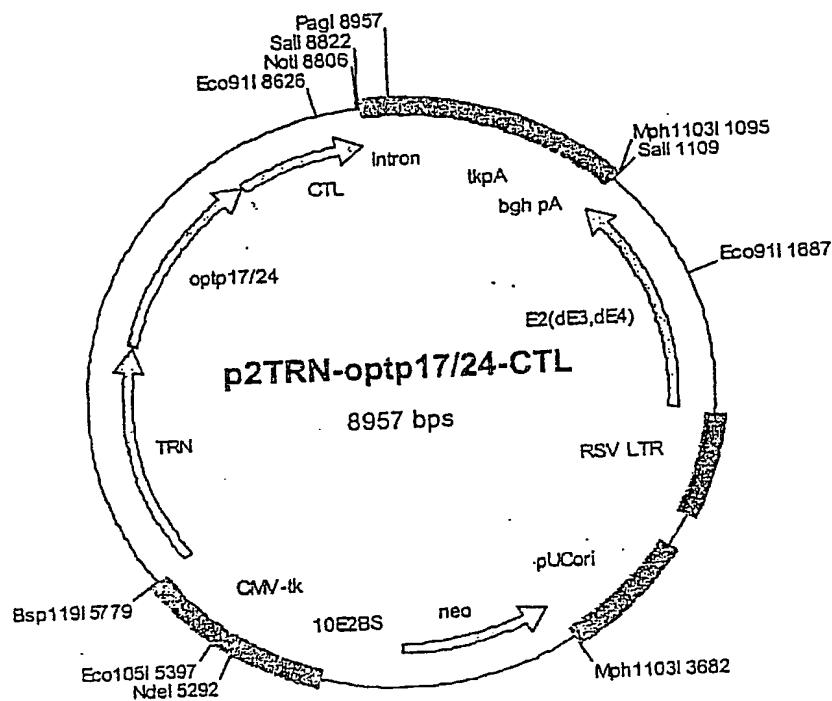


FIG. 42K

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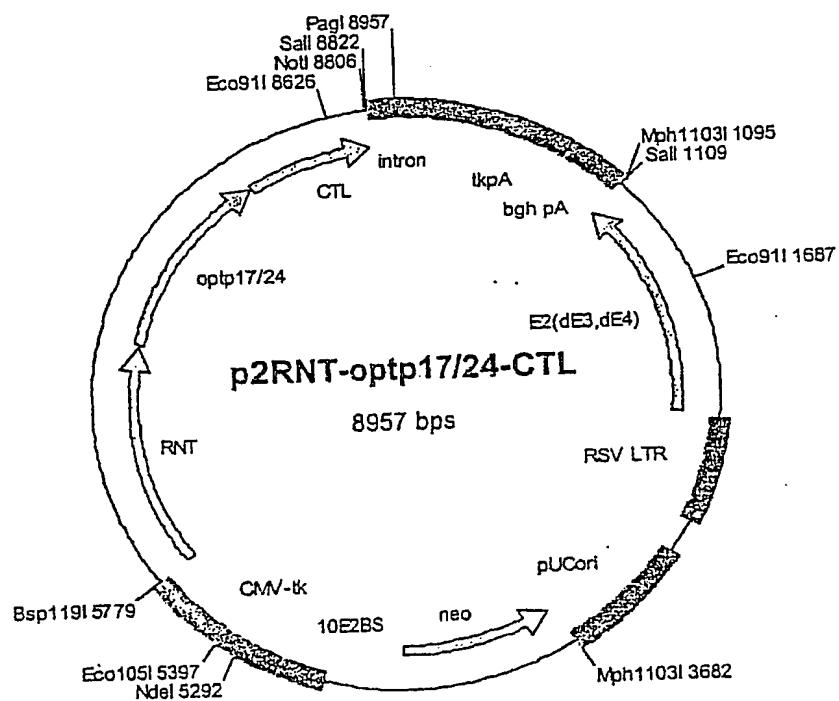


FIG. 42L

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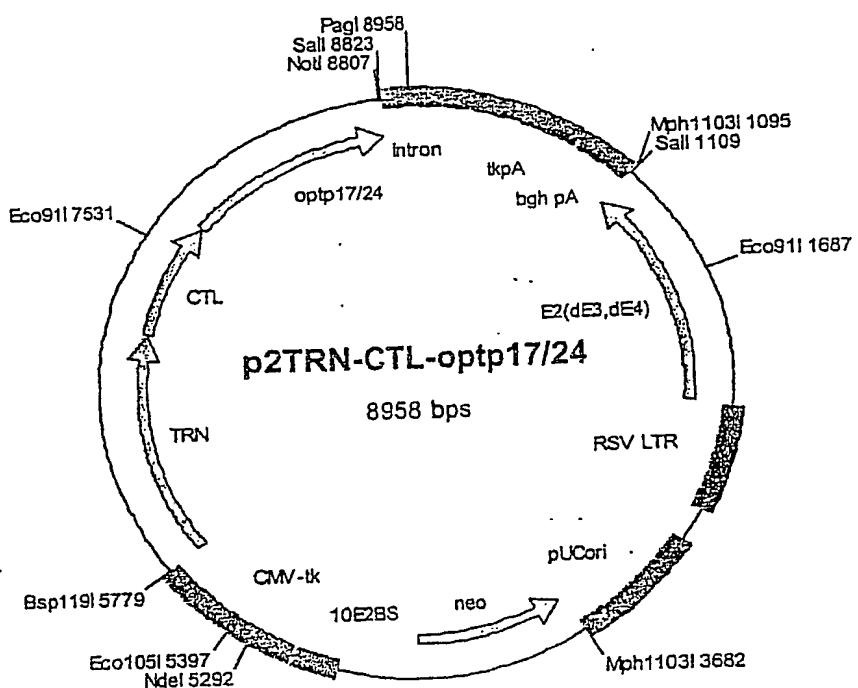


FIG. 42M

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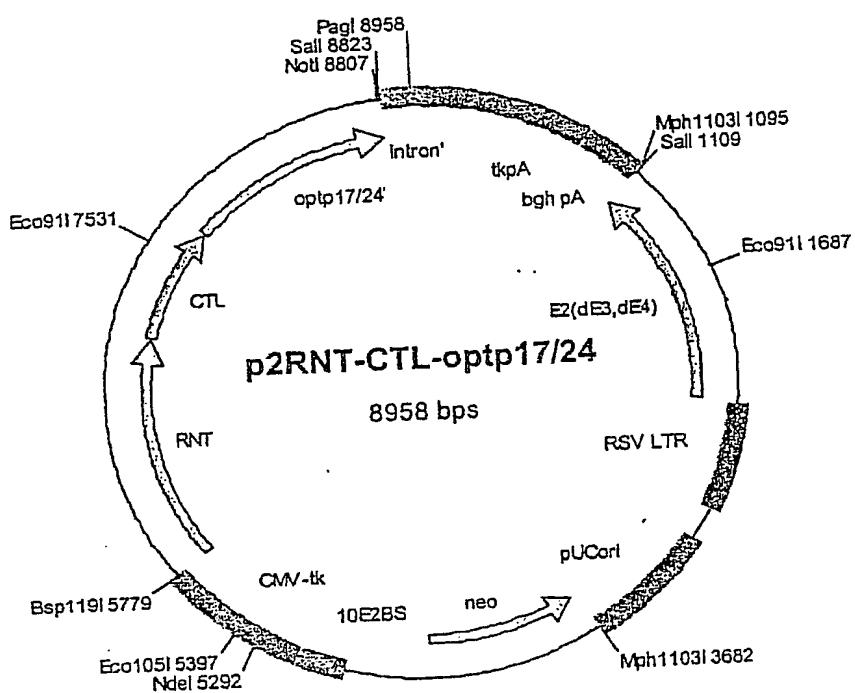


FIG. 42N

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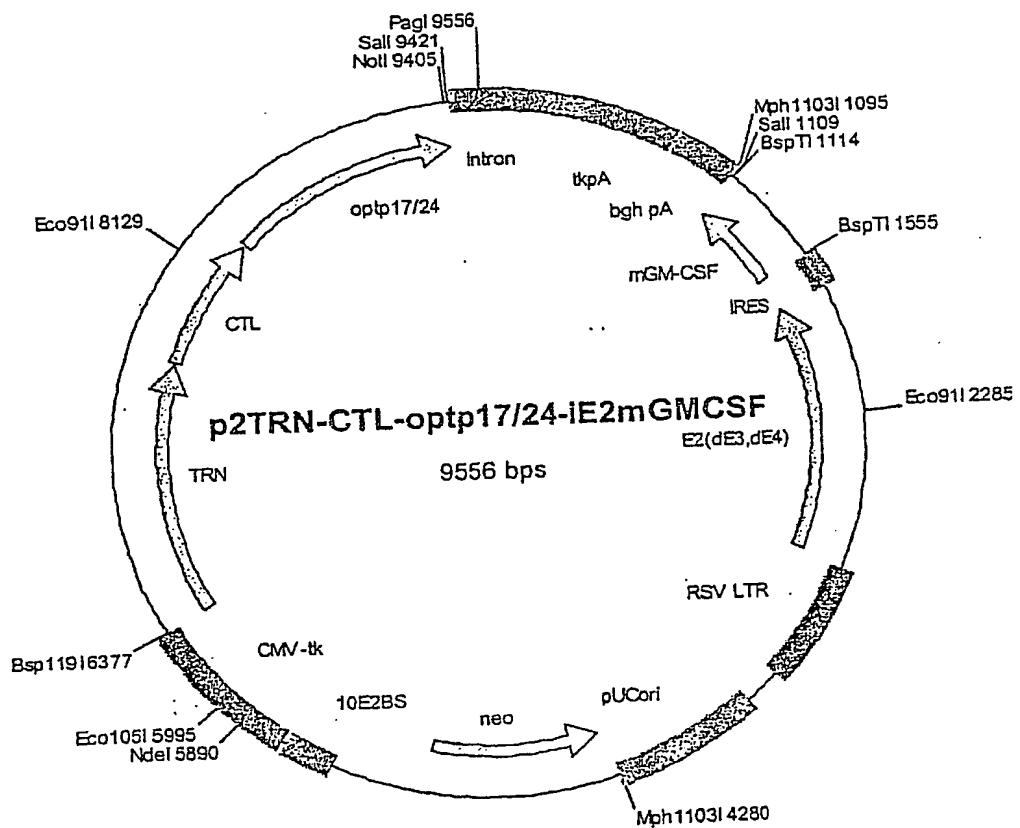


FIG. 42 O

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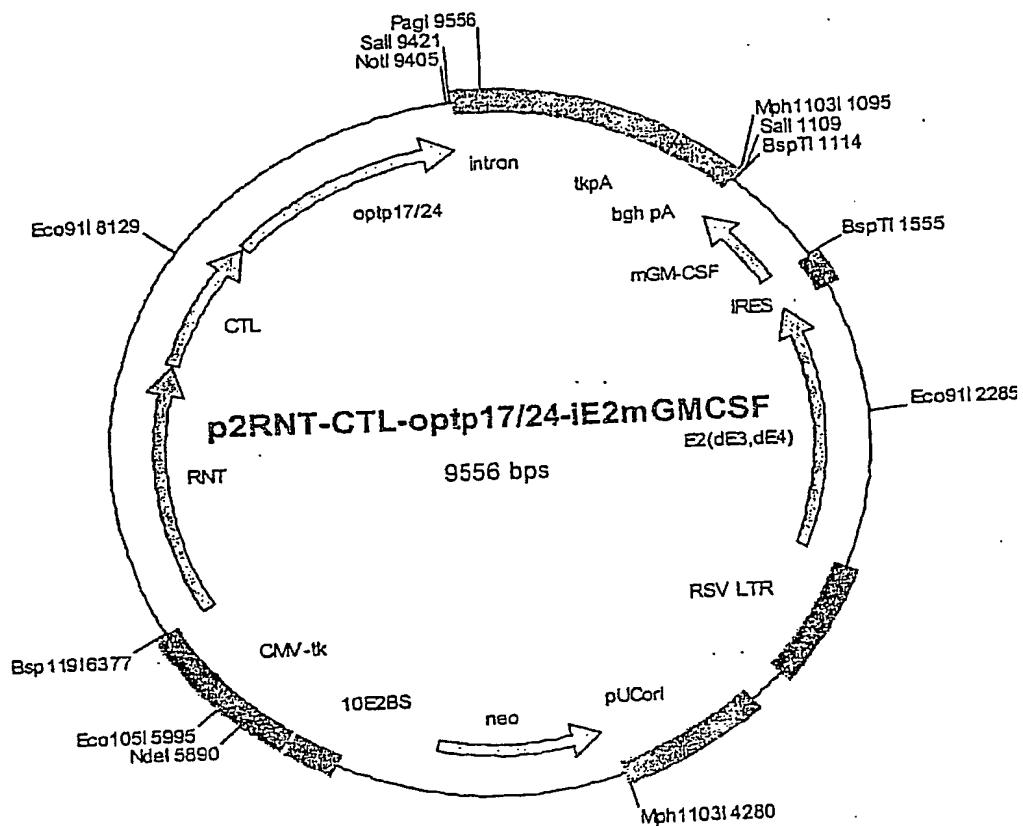


FIG. 42P

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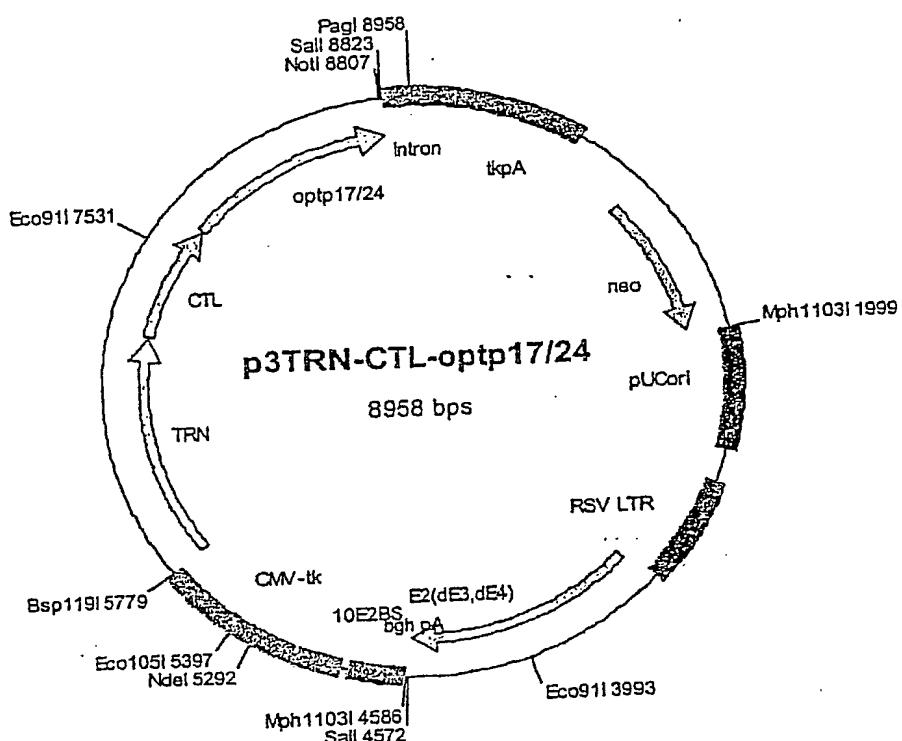


FIG. 42Q

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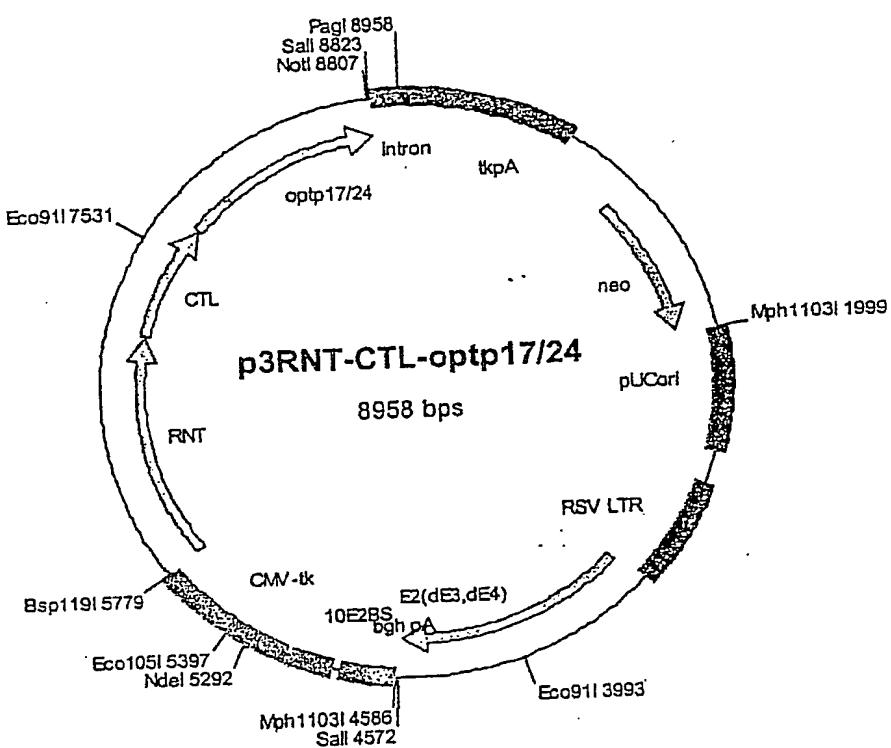


FIG. 42R

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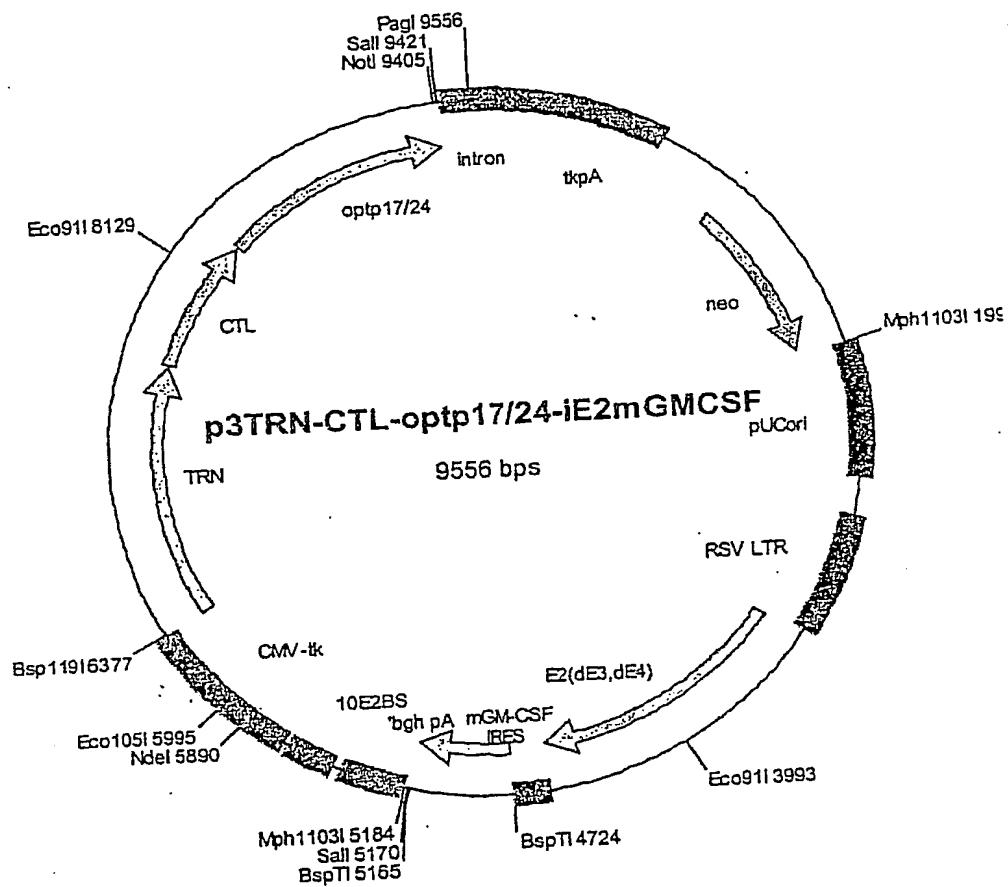


FIG. 42S

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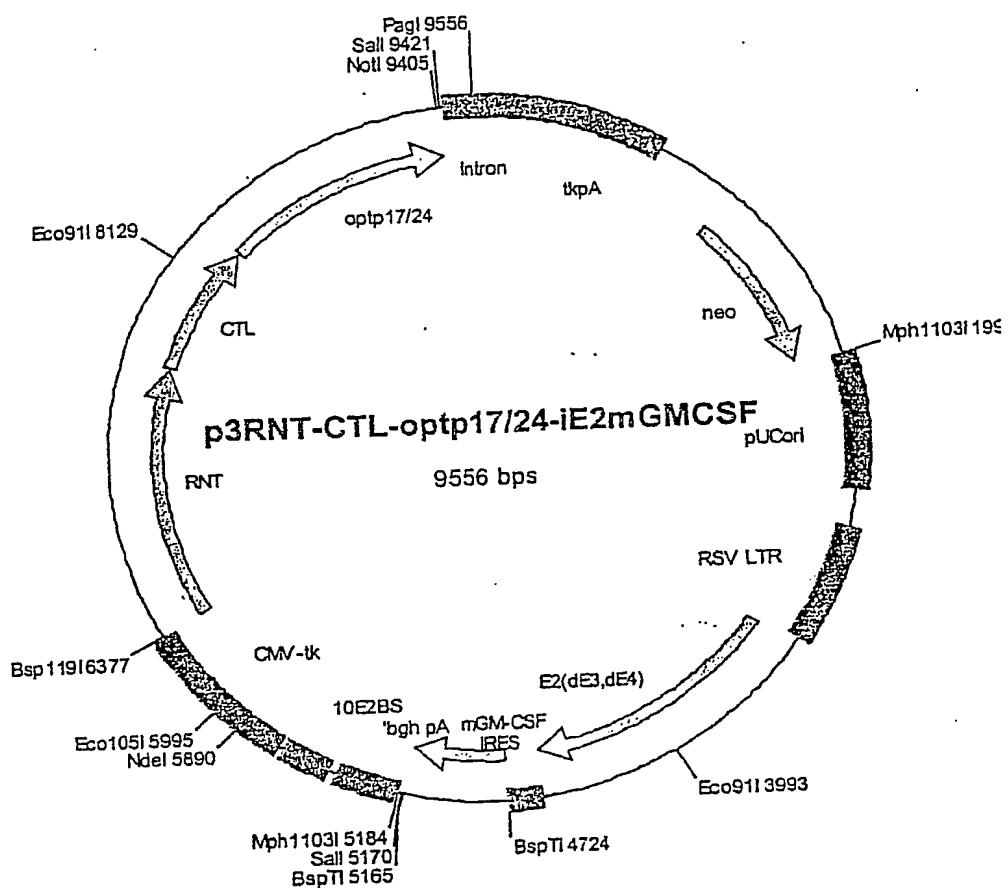


FIG. 42T

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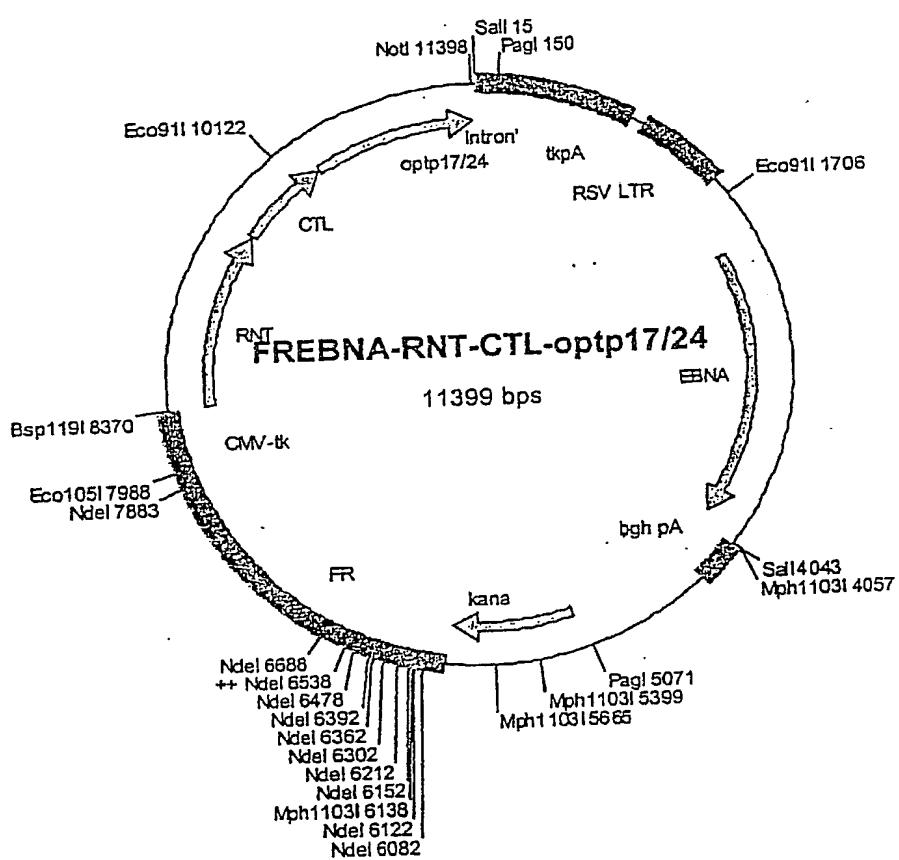


FIG. 42U

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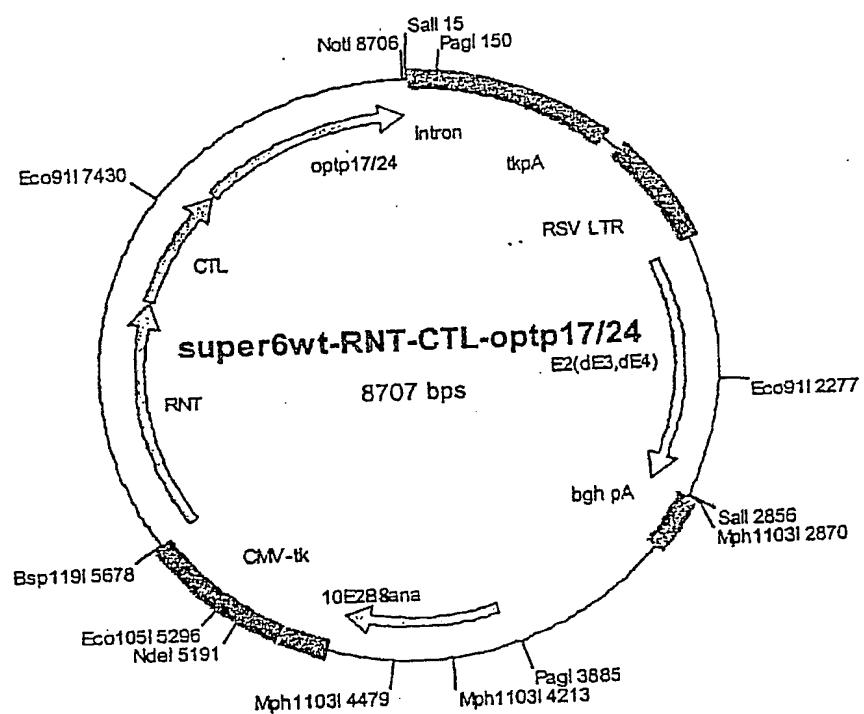


FIG. 42V

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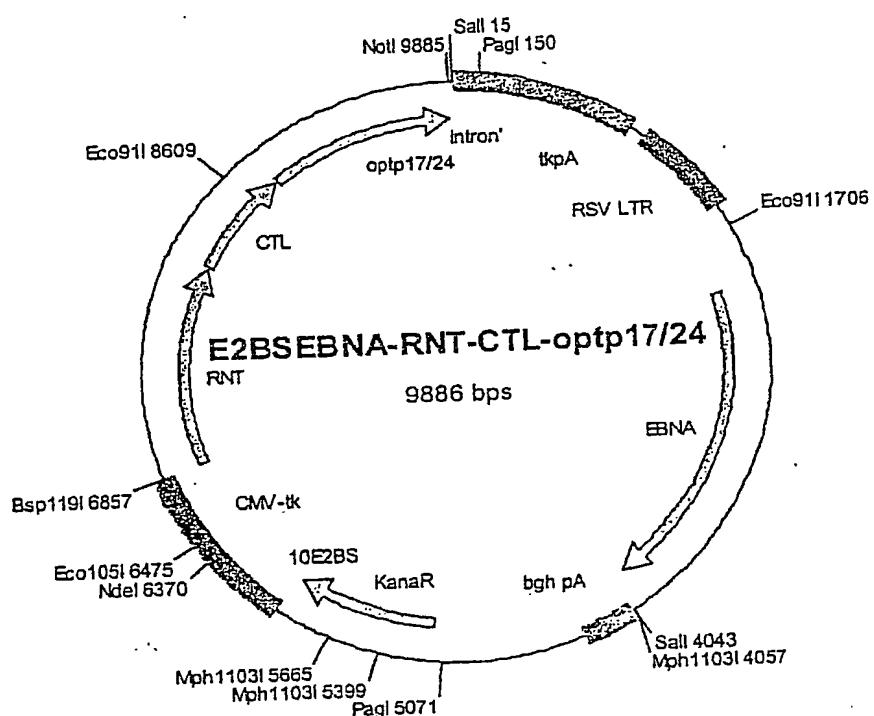


FIG. 42W

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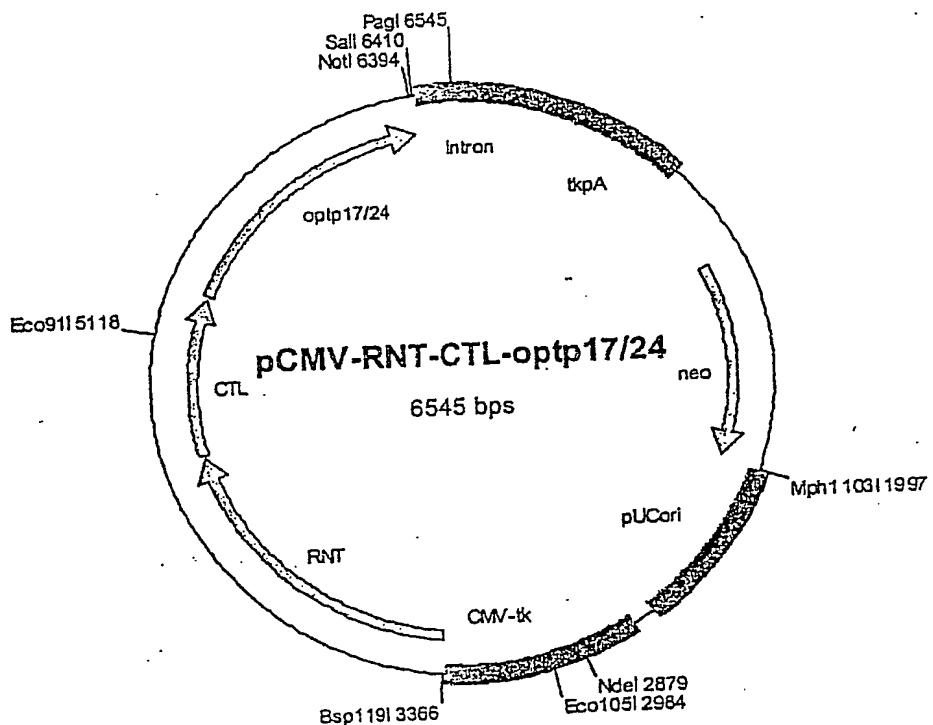


FIG. 42X

SEQUENCE LISTING

<110> FIT Biotech Oyj Plc

<120> Novel expression vectors and uses thereof

<130> 11041-006-228 (2010149)

<140> To be assigned

<141> 2002-05-03

<150> FI 20010922

<151> 2001-05-03

<160> 52

<170> PatentIn version 3.1

<210> 1

<211> 1260

<212> DNA

<213> Artificial Sequence

<220>

<223> Hybrid protein comprised of Nef-Tat-Rev (NTR)

<400> 1

atgggtggca	agtggtcaaa	atgttagtgga	tggctactg	taaggaaaag	aatgaaaacaa	60
gctgagcccg	agccagcgc	agatggggtg	ggagcagcat	ctcgagacct	ggaaaaacat	120
ggagcaatca	caagtagcaa	tacagcaact	aataacgctg	cttgtgcctg	gctagaagca	180
caagaggaa	aggaatgtgg	ttttcagtc	agacccatgg	tacctttaag	accaatgact	240
tacaaggggag	ctttagatct	tagcoactt	ttaaaaagaaa	aggggggact	ggaagggtta	300
attttactccc	caaaaagaca	agagatcctt	gatctgtggg	tctaccacac	acaaggctac	360
ttccctgttatt	ggcagaacta	cacaccaggg	ccaggggtca	gatatccact	gacctttgga	420
tggtgcttca	agttagtacc	agttgaacca	gatgaagaag	agaacagcag	cctgttacac	480
cctgcgagcc	tgcattggac	agaggacacg	gagagagaag	tgtttaatgt	gaagtttgac	540
agccatctag	catttcatca	caaggccccg	gagctgcattc	cgaggactacta	caaagactgc	600
actagtgcag	gaagaaggccg	agacagcgac	gaagagctcc	tcaagacagt	cagactcatc	660
aagtttctct	accaaagcaa	ccctcctccc	agcaacgagg	ggacccgaca	ggcccgaga	720
aatcgaagaa	gaaggtggag	agagagacag	aggcagatcc	gttcgattag	tgagcggatt	780
cttagcact	ttctggggacg	acctgcggag	cctgtgcctc	ttcagctacc	gccgcttgag	840
agacttactc	ttttagtttag	cgaatgttg	ggaaactctg	ggacgcagg	ggtgggaagt	900
cctcaagttat	ttggtggaaatc	tcctgcgtt	ttggagcccg	gaactaaaga	aaagcttgag	960
ccagtagatc	cttagactaga	gccctggaaag	catccaggaa	gtcagcctag	gaccctttgt	1020
accaattgt	attgtaaaaa	gtgttgcctt	cattgccaag	tttgcattcac	aagaaaaggc	1080
ttaggcatct	cctatggcag	gaagaaggccg	agacagcgac	gaagagctcc	tcaagacagt	1140
cagactcata	aagtttctct	accaaagcaa	ccctcctccc	agcaacgagg	ggacccgaca	1200
ggcccgaga	aatcgaagaa	gaaggtggag	agagagacag	aggcagatcc	gttcgattag	1260

<210> 2

<211> 1260

<212> DNA

<213> Artificial Sequence

<220>

<223> Hybrid protein comprised of Tat-Rev-Nef (TRN)

<400> 2

atggagccag	tagatcctag	actagagccc	tggaagcattc	caggaagtca	gcctaggacc	60
ccttgcattca	attgttattt	taaaaagtgt	tgcatttcatt	gccaagtttg	tttcacaaga	120
aaaggcttag	gcatttcata	tggcaggaaag	aaggccgac	agcgcacgaag	agctctcaa	180
gacagtccaga	ctcatcaagt	ttcttacca	aagcaaccct	cctcccaagca	acgaggggac	240
ccgacaggcc	cgaagaaaatc	gaagaagaag	gtggagagag	agacagaggc	agatccgttc	300

gatactatgt	caggaagaag	cgagacagc	gacgaagagc	tcctcaagac	agtcagactc	360
atcaagttt	tctaccaaag	caaccctct	cccagcaacg	aggggacccg	acaggccg	420
agaaatcgaa	gaagaagtg	gagagagag	cagaggcaga	tccgttgcgt	tagtgacgg	480
attcttagc	ctttctggg	acgacctgcg	gagcctgtgc	ctttcagct	accgcccgtt	540
gagagactta	ctcttgatg	tagcgaat	tgtggaaact	ctgggacgc	gggggtggga	600
agtcccaag	tatttgtga	atccctgc	gtattggagc	caggaactaa	agaaaagctt	660
gtgggcaagt	ggtcaaaatg	tagtggatgg	cctactgtaa	gggaaagaat	gaaacaagct	720
gagcctgagc	cagcagcaga	tgggtggga	gcacatctc	gagacctgg	aaaacatgg	780
gcaatcacaa	gttagcaat	agcaactaa	aacgctgtt	gtgcctggct	agaagcacaa	840
gaggaagagg	aagtgggtt	tccatcaga	cctcaggat	ctttaagacc	aatgacttac	900
aaggagctt	tagatcttag	ccactttta	aaagaaaagg	ggggactgg	agggttaatt	960
tactccccaa	aaagacaaga	gatccttgat	ctgtgggtct	accacacaca	aggctactc	1020
cctgattggc	agaactacac	accagggcca	ggggtcagat	atccactgac	ctttggatgg	1080
tgcttcaagt	tagtaccat	tgaaccat	gaagaagaga	acagcagcct	gttacaccct	1140
gcgagctgc	atgggacaga	ggacacggg	agagaagtgt	taaagtggaa	gtttgacagc	1200
catctagcat	ttcatcacaa	ggcccgagag	ctgcacatccgg	agtactacaa	agactgctga	1260

<210> 3
<211> 1260
<212> DNA
<213> Artificial Sequence

<220>
<223> Hybrid protein comprised of Rev-Tat-Nef (RTN)

<400> 3						
atggcaggaa	gaagcggaga	cagcgaacgaa	gagctctca	agacagtcag	actcatcaag	60
tttcttacc	aaagcaaccc	tcctccca	aacgagggg	ccgcacaggc	ccgaagaaat	120
cgaagaagaa	ggtggagaga	gagacagagg	cagatccgtt	cgattgttg	gcccatttt	180
agcactttt	tgggacggacc	tgcggaccc	gtgcetttc	agctaccgc	gtttgagaga	240
cttactctt	attttagcga	agatgtgg	aactctgg	cgccgggg	ggaaagtctt	300
caagtattgg	tggaatctcc	tgcagtattt	gagccaggaa	ctaaagaaac	tagtgagcca	360
gtagatctta	gactagagcc	ctgaaagcat	ccaggaatc	agcctaggac	cccttgtacc	420
aattgtctatt	gtaaaaatgt	ttgccttcat	tgcctttt	gtttcacaag	aaaaggctt	480
ggcatctctt	atggcaggaa	gaagcggaga	cagcgaacgaa	gagctctca	agacagtcag	540
actcatcaag	tttcttacc	aaagcaaccc	tcctccca	aacgagggg	ccgcacaggc	600
ccgaagaaat	cgaaagaaagaa	ggtggagaga	gagacagagg	cagatccgtt	cgataagctt	660
gtgggcaagt	ggtcaaaatg	tagtggatgg	cctactgtaa	gggaaagaat	gaaacaagct	720
gagcctgagc	cagcagcaga	tgggtggga	gcacatctc	gagacctgg	aaaacatgg	780
gcaatcacaa	gttagcaat	agcaactaa	aacgctgtt	gtgcctggct	agaagcacaa	840
gaggaagagg	aagtgggtt	tccatcaga	cctcaggat	ctttaagacc	aatgacttac	900
aaggagctt	tagatcttag	ccactttta	aaagaaaagg	ggggactgg	agggttaatt	960
tactccccaa	aaagacaaga	gatccttgat	ctgtgggtct	accacacaca	aggctactc	1020
cctgattggc	agaactacac	accagggcca	ggggtcagat	atccactgac	ctttggatgg	1080
tgcttcaagt	tagtaccat	tgaaccat	gaagaagaga	acagcagcct	gttacaccct	1140
gcgagctgc	atgggacaga	ggacacggg	agagaagtgt	taaagtggaa	gtttgacagc	1200
catctagcat	ttcatcacaa	ggcccgagag	ctgcacatccgg	agtactacaa	agactgctga	1260

<210> 4
<211> 1260
<212> DNA
<213> Artificial Sequence

<220>
<223> Hybrid protein comprised of Tat-Nef-Rev (TNR)

<400> 4						
atggagccag	tagatcttag	actagagccc	tggaaagcatc	caggaagtca	gccttaggacc	60
ccttgtacca	attgtattt	taaaaatgt	tgccttcatt	gccttttt	tttcacaaga	120
aaaggcttag	gcacatctca	tggcaggaa	aaaggcggac	agcgaacgaa	agtcctcaa	180
gacagtca	ctcataatgt	tttcttacc	aacaaacctt	cctcccaggc	acgaggggac	240
ccgacaggcc	cgaagaaatc	gaagaagaag	gtggagagag	agacagggc	agatccgtt	300
gatactatgt	tggcaagt	gtcaaaatgt	agtggatggc	ctactgtaa	ggaaagaatg	360

aaacaagctg agcctgagcc agcagcagat ggggtggag cagcatctcg agacctggaa 420
 aaacatggag caatcacaag tagacaataa gcaactata acgctgctg tgcctggcta 480
 gaagcacaag aggaagagga agtgggtttt ccagtcagac ctcaggtacc ttaagacca 540
 atgacttaca agggagctt agatcttagc cacttttaa aaaaaaggg gggactggaa 600
 gggtaattt actccccaaa aagacaagag atccttgatc tgtgggtcta ccacacacaa 660
 ggctacttcc ctgattggca gaactacaca ccaggccag gggtcagata tccactgacc 720
 tttggatgtt gcttcaagtt agtaccagtt gaaccagatg aagaagagaa cagcagctg 780
 ttacaccctg cgagctgca tggacagag gacacggaga gagaagtgtt aaagtggaaag 840
 tttgacagcc atctgatc tcatcacaag gcccggagac tgcatccgga gtactacaaa 900
 gactgcaagc ttgcaggaag aagcggagac agcgcgaaag agctccctcaa gacagtccg 960
 ctcatcaagt ttctctacca aagcaaccct cttcccaagca acgggggac ccgacaggcc 1020
 cgaagaatc gaagaagaag gtggagagag agacagagac agatccgtc gattatgtgag 1080
 cggattcttgc acacttttctt gggacgacct gggggcctg tgcccttca gctaccggcg 1140
 cttgagagac ttacttcttga ttgttagcga gattgtggaa actctggac gcaggggggtg 1200
 ggaagtccctc aagtatttgtt ggaatcttcc tcagtttgg agccaggaac taaagaatag 1260

<210> 5
 <211> 1260
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Hybrid protein comprised of Rev-Nef-Tat (RNT)

<400> 5
 atggcaggaa gaagcggaga cagcgcgaa gagctcctca agacagtca actcatcaag 60
 ttctctacc aaagcaaccc tcctccctc aacgggggaa cccgacaggc ccgaagaaat 120
 cgaagaagaa ggtggagaga gagacagagg cagatccgtt cgatttagtga ggggatttt 180
 agcaacttttc tgggacgacc tgccggccct gtgccttcc agctaccggc gcttggaga 240
 cttaactcttgc atgttagcga agatgtggaa aactctggaa cgcgggggtt gggaaagtctt 300
 caagtattttgg tggaaatctcc tgcaatgttgg gggccggaa ctaaaagaaac tagtgtggc 360
 aagtggtcaa aatgttagtgg atggctact gtaaggaaa gaatgaaaca agctggcct 420
 gagccagcag cagatgggggtt gggggcggca ttcggggggc tggggaaaaca tggggcaatc 480
 acaagttagca atacagcaac taataacgct gottgtgeet ggcttagaagc acaagaggaa 540
 gaggaaatgttgg gttttccatc cagacccatc gtaccccttac gaccaatgac ttacaaggaa 600
 gttttatggatc tttagccatc tttaaaagaa aaggggggac tgggggggtt aattttactcc 660
 ccaaaaagac aagagatcct tgatctgtgg gtcattaccaca cacaaggctt cttccctgtat 720
 tggcagaact acacaccagg gccaggggtc agatatatccat tgacccttgg atgggtgttc 780
 aagtttagtac cagttgaaacc agatgaagaa gagaacagca gcttggatca ccctgcgagc 840
 ctgcattggaa cagaggacac ggagagagaa gtgtttaatgtt ggaagtttgc cagccatcta 900
 gcatttccatc acaaggcccg agagatgcatttccgggactt acaaaagactt cagcttgag 960
 ccagtagatc cttagactaga gccctggaa catccggaa gtcagcttgc gacccttgc 1020
 accaattgtt attgtaaaaaa gtgtggcctt cattggccaaat tttgtttcac aagaaaaggc 1080
 tttaggcattc cctatggcag gaagaagcgg agacagccac gaaagactcc tcaagacatg 1140
 cagactccatc aagtttcttcc accaaagcaa ccctccctcc agcaacgggggggggggac 1200
 gggccggaa aatcgaagaa gaagggtggag agagagacag aggcagatcc gttcgattag 1260

<210> 6
 <211> 1164
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Protein comprised of Immunodominant parts of the Nef-Tat-Rev (NTR)

<400> 6
 atgggatggc ctactgttaag ggaaagaatg aaacaagctg agcctgagcc agcagcagat 60
 ggggtggggag cagcatctcg agacctggaa aaacatggag caatcacaag tagcaatata 120
 gcaactataa acgctgcttgc tgcctggcta gaagcacaag aggaagagga agtgggtttt 180
 ccagtcagac ctcaggtacc ttaagacca atgacttaca agggagctt agatcttagc 240
 cacttttaa aaaaaaggg gggactggaa gggtaattt actccccaaa aagacaagag 300
 atccttgatc tggggctca ccacacacaa ggctacttcc ctgattggca gaactacaca 360
 ccaggccag gggtcagata tccactgacc ttggatgtt gcttcaagtt agtaccagtt 420

gaaccagatg	aagaagagaa	cagcagccgt	ttacaccctg	cgagccgtca	tgggacagag	480
gacacggaga	gagaagtgtt	aaagtggaaag	tttgacagcc	atcttagcatt	tcatcacaag	540
gccccggagc	tgcataccgg	gtactacaaa	gactgcgc	tggccggcgt	tgagccagta	600
gatccatagac	tagagccctg	gaagcatcca	ggaaggctc	cttaggacccc	ttgttacaat	660
tgttatgtt	aaaagtgtt	ccttcattgc	caagtttgtt	tcacaagaaa	aggtttaggc	720
atctccatag	gcagggaaag	gcccggagac	cgacgaaagag	cttcctaaga	cagtcaagact	780
catcaagttt	cttcacccaa	gaaaccctcc	tcccaagcaac	gaggggaccc	gacaggcccc	840
aagaatccg	gactggccat	cctgtctgagc	gacgaagagc	tcctcaagac	agtcaagactc	900
atcaagtttc	tctacccaaag	caaccctcct	cccgaccaacg	aggggacccg	acaggcccc	960
agaaaaatcgaa	gaagaagggt	gagagagaga	caaggccaga	tccgttgcgt	tagtgagccgg	1020
atttcttagca	ctttttctgg	acgacactcg	gagcctgtgc	cttcttcagct	accggccctt	1080
gagagactta	ctcttgattt	tagcgaagat	tgtggaaact	ctgggacgca	gggggtggga	1140
agtccctcaaq	tatttgttga	atga				1164

```
<210> 7
<211> 1173
<212> DNA
<213> Artificial Sequence
```

<220>
<223> Protein comprised of Immunodominant parts of the Nef-Tat-Rev
separated by protease sites(NTR)

```
<210> 8
<211> 1161
<212> DNA
<213> Artificial Sequence
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<220>
<221> Protein comprised of Immunodominant parts of the regulatory proteins
Nef-Tat-Rev started from aal of Nef (N11TR)

acggagagag	aagtgttaaa	gtggaagttt	gacagccatc	tagcattca	tcacaaggcc	540
cgagagctgc	atccggagta	ctaccaaagac	tgcgctctgg	ccgcccgttg	gccagtagat	600
ccttagactag	agccctggaa	gcatccagga	agtcagccata	ggacccttg	taccaattgc	660
tattgttaaa	agtgttgccct	tcatggccaa	gttggttca	caagaaaagg	cttaggcac	720
tccttatggca	ggaagaagcg	gagacagcga	cgaagagctc	ctcaagacatc	tcagactcat	780
caagtttctc	taccaaagca	accctcctcc	cagcaacag	gggaccgcac	aggccccgaa	840
aaatccggac	tggecatct	gctgagcgac	gaagagctcc	tcaagacatc	cagactcatc	900
aagtttctct	accaaagcaa	ccctccccc	agcaacgagg	ggaccgcaca	ggcccgaaga	960
aatcaagaa	gaagggtggag	agagagacag	aggcagatcc	gttcgattag	tgagcggatt	1020
cttagcactt	ttctgggacg	acctgcggag	cctgtgcctc	ttcagctacc	gccgctttag	1080
agacttactc	ttgatgttag	cgaagatttg	ggaaactctg	ggacgcagg	ggtggaaagt	1140
cctcaagat	tgggaaatg	a				1161

<210> 9
<211> 1170
<212> DNA

<213> Artificial Sequence

<220>
<223> Protein comprised of Immunodominant parts of the regulatory proteins Nef-Tat-Rev started from aal of Nef separated by protease sites (N11TR)

<400>	9					
atgtggcccta	ctgttaaggga	aagaatgaaa	caagctgagc	ctgagccagc	agcagatggg	60
gtgggagcag	catctcgaga	cctggaaaaa	catggagcaa	tcacaagtag	caatacagca	120
actaataaac	ctgttgtgc	ctggtagaa	gcacaagagg	aagaggaatg	gggttttcca	180
gtcagaccc	aggtaacctt	aagaccaatg	acttacaagg	gagcttttaga	tcttagccac	240
tttttaaaag	aaaagggggg	actggaaggg	ttaattttact	ccccaaaaag	acaagagatc	300
cttgatctgt	gggtctacca	cacacaaggc	tacttccctg	attggcgaaaa	ctacacacca	360
gggcaggggg	tcagatatcc	actgacccctt	ggatgggtgt	tcaagtttagt	accagttgaa	420
ccagatgaag	aagagaacag	cagctgttta	ccccctcgca	gcctgcattgg	gacagaggac	480
acggagagag	aagtgttaaa	gtggaagttt	gacagccatc	tagcattca	tcacaaggcc	540
cgagagctgc	atccggagta	ctaccaaagac	tgcgctctgg	ccttcaagcg	ggtgtgagoca	600
gtagatccca	gactagagcc	ctgaaagcat	ccaggaagtc	agccttaggac	cccttgcattc	660
aattgtctt	gtaaaaaaatg	ttgccttcat	tgccaaatgg	gtttcacaag	aaaaggctta	720
ggcatctct	atggcaggaa	gaaggggaga	cagcgacgaa	gagctctca	agacagtcag	780
atccatcaag	tttctctacc	aaagcaaccc	tcctcccgac	aaagggggg	cccgacaggc	840
ccgaagaaat	ccgtacggg	gaaggcgctg	ctgagcgacg	aagagtcct	caagacatc	900
agactcatca	agtttctcta	ccaaagcaac	cctcctccca	gcaacgggg	gaccgcacag	960
gccccaaagaa	atcgaagaag	aagggtggaga	gagagacaga	ggcagatccg	ttcgattagt	1020
gagcggat	ttagcactt	tctgggacga	cctgcggagc	ctgtgcctct	tcagctaccg	1080
ccgcattgaga	gacttactct	tgatgttag	gaagatttg	ggaaactctgg	gacgcagg	1140
gtgggaaatgc	ctcaagttt	gggtggaaatg	a			1170

<210> 10
<211> 663
<212> DNA
<213> Artificial Sequence

<220>
<223> Protein comprised of Cytotoxic T-cell epitopes of Pol and Env genes (CTL)

<400>	10					
atgatcaccc	tgtggcagcg	ccccctggtg	gccctgtatcg	agatctgcac	cgagatggag	60
aaggaggcca	agatcagaa	gatccggcccc	gcggccctga	agaagaagaa	gagcgtgacc	120
gtgctggacg	tgggcgacgc	ctacttcagc	gtgccccctgg	ataaggactt	ccgcaagtac	180
accgccttca	ccatccccag	catctggaaag	ggcagccccg	ccatcttcca	gagcagcatg	240
accaagaagc	agaaccccg	catctgtatc	taccaatata	tggacgacct	gtacgtgccc	300
atcgtgtgc	ccgagaagga	cagctggctg	gtgggcaagc	tgaactgggc	cagccagatc	360
tacgcggcca	tcaagggtgaa	gcagctgatc	ctgaggagac	ccgtgcacgg	cgtgtacgag	420
cccatctgtgg	gcccggagac	cttctacgtg	gacggcgcccg	ccaaaccgcgc	ccgcaactcg	480
ttggtgaccg	tgtactacgg	cgtggccctgt	tggaggagg	ccaccaccac	cctggtgag	540

cgctacctgc	gacaccagca	gctgctggc	atctgggct	gcgcctgac	cccctacgac	600
atcaaccaga	tgctgcgg	ccctggccgc	gccttcgtga	ccatccgcca	ggcagcctg	660
tag						663

<210> 11
<211> 1266
<212> DNA
<213> Artificial Sequence

<220>
<223> Truncated Gag protein sequence (dgag)

<400> 11	atgttagaca	aatggaaaaa	aattcggta	aggccagggg	gaaagaaaaa	atatcaatta	60
	aaacatata	tgatggcaag	caggagcta	gaacgattcg	cagttatcc	tggcctgtt	120
	gaaacatca	aaggctgt	tag acagataat	ggacagctac	aaccgtcc	tcagacagga	180
	tcagaagaa	cttagatcatt	atataataca	gtaccaaccc	tctattgtgt	gcatcaaaag	240
	atagaggtaa	aagcacccaa	ggaagctt	gacaaggtag	aggaagagca	aaacaacagt	300
	aagaaaaagg	cacagcaaga	agcactgtac	gcaggaaaca	gaaaccagg	cagccaaaat	360
	taccctatag	tgccaaacct	acaggacaa	atggatcatc	aggccatatc	acctagaact	420
	ttaaatgc	atggaaaaat	agtggaaagag	aaggcttca	gcccagaagt	aataccatg	480
	ttttcagcat	tatcagaagg	agccaccccc	caagatttaa	acaccatgt	aaacacagt	540
	gggggacat	aagcaggccat	gcaaatgtt	aaagaaaaacca	tcaatgagga	agctgcagaa	600
	tgggatagat	tgcacccagt	gcatgcgg	cttattgcac	caggccat	gagagaacca	660
	aggggaatg	acatgcagg	aactactgt	acccttcagg	aacaatagg	atggatgaca	720
	aataatccac	ctatccagt	aggagaaaata	tataagat	ggataatct	gggattaaat	780
	aaaatagtaa	aatgtatag	ccctaccagc	attctggata	taaaacaagg	acccaaagaa	840
	ccctttagag	attatgtaga	ccggttctat	aaaaccctaa	gagccgagca	agctacacag	900
	gaagtaaaa	attggatgc	agaaacccat	tttgtccaa	atgcgaatcc	agattgtaa	960
	actatTTTA	aaggatttag	accaggact	aactagaag	aatatgtac	agcatgtcag	1020
	ggagtgggg	gaccggcca	taaagcaaga	tttttggctg	aagcaatgag	ccaagtaaca	1080
	gtttcagctg	ccataatgtat	gcagagaggc	aatttttaga	accaaagaaa	gactgttaag	1140
	tgttcaatt	gtggcaaaaga	agggcacata	gcagaaaaat	gcagggcccc	tagaaaaaag	1200
	ggctgttgg	aatgtggaaa	ggaaggacat	caaataagg	attgcacaga	aagacaggct	1260
	aattag						1266

<210> 12
<211> 1092
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic coding sequence for p17/24 protein of Gag gene (syn 17/24)

<400> 12	atgggcgcaa	gaggctccgt	gctgagccgc	ggagagctgg	acaagtggga	gaagatccgc	60
	ctgcgc	ccgc	gcccggaa	gaatgtaccag	ctgaaggaca	tcgtgtggc	120
	ctggagcg	ct	ccggccctg	ctcgagacca	gcaaggctg	ccgcccagatc	180
	atggcc	cac	ccac	ctccac	ggcagcgcgg	agctgcgcag	240
	accgtggcc	ccctgtact	ctgtgcac	aaatgcagg	tgaaggacac	caaggaggcc	300
	ctggaca	aa	gg	ggaggagga	gcaacaac	agaagaaga	360
	gacgcgc	acc	cc	ggtagccag	aactacccc	tgctgcagaa	420
	catatgtgc	accaggccat	cagcccccc	accctgaac	cctgggtgaa	gtgtgtggag	480
	gagaaggc	ct	cc	ggatcc	atgttcagcg	ccctgagcga	540
	ccccaggacc	tgaacaccat	gctgaacacc	gtggggcc	accaggccgc	catgcagat	600
	ctgaaggaga	ccatcaac	gaggccgc	gatgtggacc	gcctgcaccc	ctgtgcacgc	660
	ggcccatcg	ccccggcc	gatgcgcg	ccccggc	gctgcaccc	cgccacacc	720
	agcaccc	aggagc	cggtggat	accaacaacc	ccccatccc	ctgtggcg	780
	atctaca	ac	cctggccct	aacaagatcg	tccgcat	cagccccacc	840
	agcattct	acatca	ggggccca	gagcccttcc	gctgcacat	ggaccgc	900
	tacaagac	tgcgccg	gaggccacc	caggagg	gaaactggat	gaccgagacc	960
	ctgctgt	agaacg	ccca	ccccactgc	aagaccatcc	tcaaggccct	1020
	gccacc	ctgg	aggat	gaccgc	caaggcgtgg	ggggcccccgg	1080

cgcgtgtgt ga 1092

<210> 13
<211> 1092
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic coding sequence for p17/24 protein of Gag gene optimized for expression in eukaryotic cells (optp17/24)

<400> 13
atgggcgaa gagcctccgt gctgagcggc ggagagctgg acaagtggga gaagatccgc 60
ctgcgcggc gcggaacaaa gaagtaccag ctgaaggcaca tcgtgtggc cagccgcgag 120
ctggagcgct tcgcgtgaa ccccgccctg ctgcgaccca gegaaggctg cggccagatc 180
atggccgcggc tccaggccag ccttcagacc ggcagcgggg agtgcgcgag cctgtacaac 240
accgtggcca ccctgtactg cgtgcaccagg aagatcgagg tgaaggacac caaggaggcc 300
ctggacaagg tggaggagga gcagaacaac agcaagaaga aggoccagca ggaggccgccc 360
gacgcggcga accgcaacca agtcagccag aactacccca tcgtgcagaa cctgcaggcc 420
cagatggtgc accaggccat cagccccccg acccctgaacg cttgggtgaa ggtggtgag 480
gagaaggccct tcagcccgaa ggtgtatcccc atgttcagcg ccttgaggcga gggcgctacc 540
ccccaggacc tgaacaccat gctgaaaccacc gtggccggcc accaggccgc catgcagatg 600
ctgaaggaga ccatcaacga ggaggccgccc gatgtgggacc gctgcacccc cgtgcacgccc 660
gggccccatcg ccccccggcca gatgcgcgag ccccgccgca ggcacatcgc cggcaccacc 720
agcacccctcc agggacgatc cggcggatc accaacaacc ccccatccc cgtggcgag 780
atctacaacgc gttggatcat cttggccctg aacaagatcg tccgcatgtc cagcccccacc 840
agcatcctgg acatcaagca gggccccaag gagcccttc ggcactacgt ggaccgcttc 900
tacaagaccc tgegcgcccga gcaggccacc caggaggtga agaactggat gaccgagacc 960
ctgctggtgc agaaccccaa ccccgacttc aagaccatcc tcaaggccct gggaccggcc 1020
gccaccctgg agggatgtat gaccgcctgc caaggcgtgg gggcccccgg ccacaaggcc 1080
cgctgtgtgt ga 1092

<210> 14
<211> 1926
<212> DNA
<213> Artificial Sequence

<220>
<223> Hybrid protein cds comprised of Tat-Rev-Nef and CTL (TRN-CTL)

<400> 14
atggagccag tagatccatg actagagccc tggaaagcatc caggaagtca gccttaggacc 60
ccttgcacca attgctattt taaaaagtgt tgcccttatt gccaagttt tttcacaaga 120
aaaggcttag gcatctccta tggcaggaag aagcggagac agcgacgaa agctcctcaa 180
gacagtcaaga ctcatcaatgt ttctcttacca aagcaaccct cctcccaagca acgaggggac 240
ccgacaggccc cgaagaatac gaagaagaag gtggagagag agacagaggc agatccgttc 300
gatactatgtc cagggaaag cggagacagc gacgaagacg tcctcaagac agtcagactc 360
atcaagttt tctacccaaag caacccttcc cccagcaacg agggggccccc acaggcccga 420
agaaatcgaa gaagaagggtg gagagagaga cagaggcaga tccgttcgat tagtgacgg 480
attcttagca ctttctggg acgacccctgcg gaggcgtgtc ctcttcagct accggcgctt 540
gagagactt ctcttgatgt tagcgaatgt tggtggaaact ctggggccgca ggggggtggga 600
agtcctcaatgt tattgtggaa atcttcgtca gatgtggggc cggggactaa agaaaaaggctt 660
gtggggcaatgt ggtcaaaatgt tagtggatgg cctactgtaa gggaaaagaat gaaacaagct 720
gaggcctgagc cagcagcaga tgggggtggga gcagcatctc gagacctggaa aaaacatggaa 780
gcaatcacaa gtacaaatac agcaactaat aacgctgttt gtgcctggct agaaggccaa 840
gagggaggaa aagtgggtt tccagtcaga cctcaaggatc cttaaagacc aatgacttac 900
aaggggatctt tagatcttag ccactttta aaagaaaaagg gggggactggaa agggtaatt 960
tactcccaa aaagacaaga gatccctgtat ctgtgggtct accacacaca aggtacttc 1020
cctgattggc agaactacac accaggccca gggggatcatgt atccactgac ctttggatgg 1080
tgcttcagtt tagtaccatgt tgaaccatgt gaagaagaga acagcggcc tttacaccct 1140
ggcggccctgc atggggacaga ggacacggag agagaaggat taaagtggaa gtttgacagc 1200
catcttagcat ttcatcacaa ggcccgagag ctgcattccgg agtactacaa agactgcgcg 1260
ggcgtcatca ccctgtggca ggcggccctg gtggccctga tcgagatctg caccggatg 1320

gagaaggagg gcaagatcg	caagatcgcc	cccgcggcc	tgaagaagaa	gaagagcgtg	1380
accgtgctgg acgtggcg	cgcctacttc	agcgtgcccc	tggataagga	cttccgc	1440
tacaccgcct tcaccatccc	cagcatctgg	aaggcagcc	ccgccccatctt	ccagagc	1500
atgaccaaga agcagaaccc	cgacatcg	atctaccagt	acatggac	cctgtacgt	1560
cccatcg	tgcccgagaa	ggacagctgg	ctggggca	agctgaactg	1620
atctacgcgc	gcatcaaggt	gaaggcagctg	atccctgaagg	agcccg	1680
gagcccatcg	tggcgccg	gaccttctac	gtggacggcg	cgcccaacc	1740
ctgtgggtga	ccgtgtacta	cgccgtgccc	gtgtggaa	aggccaccac	1800
gagcgttacc	tgcgcgacca	gcagctgt	ggcatctgg	gtgcgcctg	1860
gacatcaacc	agatgtcg	cggccctggc	cgcgccttc	tgaccatccg	1920
ctgttag				ccagggc	1926

<210> 15
<211> 1926
<212> DNA

<213> Artificial Sequence

<220>
<223> Hybrid protein cds comprised of Rev-Nef-Tat and CTL (RNT-CTL)

<400> 15					
atggcaggaa gaagcg	gaga cagc	gagctcc	ta agac	agtca	60
tttcttctacc aaagcaaccc	tcctcc	caggc	cccgac	ggcc	120
cgaagaaga a	gttggagaga	gagacagagg	cagatcc	cgattagt	180
agactt	tgggac	gacc	gtgc	cctt	240
cttactctt	at	ttgt	at	ctgg	300
caagtattt	ttgg	aatctcc	tgca	gttgg	360
aagtgtt	caa	atgt	atgg	ttgg	420
gaggcag	ca	gatgg	gggc	gggg	480
acaagtag	ta	atag	aaac	gttgc	540
gaggaa	gtt	ttcc	cagt	gttgc	600
gttttagatc	ttag	ccactt	ttt	aaagg	660
ccaaaa	agac	aagat	ttt	gggtt	720
tggcaga	act	acac	ttt	ttt	780
aagttagt	ac	ttgt	ttt	ttt	840
ctgc	atgg	gaa	ttt	ttt	900
gcattt	catc	aca	ttt	ttt	960
ccagtagatc	ctagact	aa	ttt	ttt	1020
accaattgt	attgt	aaa	ttt	ttt	1080
tttaggcattc	cctat	ttgt	ttt	ttt	1140
cagacttcatc	ttttt	ttt	ttt	ttt	1200
ggcccaaga	aat	cgaa	ttt	ttt	1260
gccc	gaa	gggt	ttt	ttt	1320
gccc	gaa	gggt	ttt	ttt	1380
gagaaggagg	gca	aa	ttt	ttt	1440
accgtgctgg	acgt	ggc	ttt	ttt	1500
tacaccgcct	tcaccat	ccc	ttt	ttt	1560
atgaccaaga	agc	aa	ttt	ttt	1620
cccatcg	tc	cc	ttt	ttt	1680
atctacgcgc	gcat	aa	ttt	ttt	1740
gagcccatcg	tgg	ggc	ttt	ttt	1800
ctgtgggtga	ccgt	gtt	ttt	ttt	1860
gagcgttacc	tgc	ggc	ttt	ttt	1920
gacatcaacc	agat	gtcg	ttt	ttt	1926
ctgttag					

<210> 16
<211> 2529
<212> DNA
<213> Artificial Sequence

<220>
<223> Hybrid protein cds comprised of Tat-Rev-Nef and truncated Gag protein (TRN-dgag)

<400> 16

atggagccag	tagatccat	actagagccc	tggaagcattc	caggaagtca	gcctaggacc	60
ccttgcacca	attgttattt	taaaaagtgt	tgccttcatt	gccaagttt	tttcacaaga	120
aaaggcttag	gcatctcta	tggcaggaa	aagcggagac	agcgacgaa	agctccctaa	180
gacagtca	ctcatca	ttcttacca	aagcaaccct	cctccagca	acgagggac	240
ccgacaggcc	cgaagaaatc	gaagaagaag	gtggagagag	agacagaggc	agatccgttc	300
gatactatgt	caggaagaag	cggagacagc	gacaaagagc	tcctcaagac	agtca	360
atcaagttt	tctaccaa	caaccctct	ccccagcaacg	aggggacc	acaggcccga	420
agaaatcgaa	gaagaatgg	gagagagaga	caaggcaga	tcggat	tagtgagcgg	480
attcttagca	cttcttgg	acgacotcg	gagcctgtc	ctcttcag	accggccgtt	540
gagagactta	cttcttgc	taggaaagat	tgtggaaact	ctgggacgca	gggggtggg	600
agtccctcaag	tattttgtt	atctctgc	gtattttgg	caggaactaa	agaaaagctt	660
gtggcgaatgt	ggtcaaaat	tagtggatgg	ctactgtaa	gggaaagaat	gaaacaagct	720
gagcctgagc	cagcagcaga	tgggttgg	geacatctc	gagacctgg	aaaacatgga	780
gcaatcacaa	gtgcaatac	agaacaaat	aacgtctt	gtgccttgg	agaagcacaa	840
gaggaagagg	aagtgggtt	tccatcaga	cctcaggat	ctttaagacc	aatgacttac	900
aaggggctt	tagatctt	ccactttt	aaagaaaagg	ggggacttgg	agggttattt	960
tactcccaa	aaagacaaga	gatccctt	ctgtgggt	accacacaca	aggctacttc	1020
ccttgcatttgc	agaactacac	accaggcc	ggggat	atccactgac	ctttggatgg	1080
tgctca	atgttacatgt	tgaaacat	gaagaagaga	acagcagc	tttacaccct	1140
gcccgcctgc	atgggacaga	ggacacgg	agagaatgt	taatgtgg	gtttgacagc	1200
catcttagcat	ttcatcaca	ggcccgag	ctgcatttgc	agtactacaa	agactgcgc	1260
gcccgtttag	acaaatgg	aaaattcg	ttaaggcc	ggggaaagaa	aaaatatcaa	1320
ttaaacata	tagatgg	aagcagg	ctagaacat	tcgcaggta	tcctggctg	1380
ttagaaacat	cagaagg	tagacat	atggacagc	tacaacccgt	ccttcagaca	1440
ggatcaga	aacttagatc	attatataat	acatgt	ccctcttattt	tgtgcata	1500
aagatagagg	taaaagacac	caaggaag	ttagaca	tagaggaa	gcaaaacaa	1560
agtaaaaa	aggcacagca	agaagcag	gacgcagg	acagaaacc	gttcaggcc	1620
aattaccctt	tagtgc	aaatgg	cttacagg	aaatgg	atcaggccat	1680
actttaat	catgg	aaaatgtt	atgtgg	gagaatgg	tcagcc	1740
atgtttt	cattatc	aggagcc	ccacaag	taaacat	gtaataacc	1800
gtgggggac	atcaagc	catgcaat	ttaaaa	ccatcaat	ggaagct	1860
gaatgggata	gattgcac	agtgc	ggcctt	caccaggcc	gatggagaa	1920
ccaaggggaa	gtgacat	aggact	atacc	aggaaacaa	aggatggat	1980
acaatataatc	cacat	atggag	atataa	gatggata	cctggatt	2040
aataaaatag	taagaatgt	tagcc	acattt	atataaa	aggacaaa	2100
gaacccttta	gagattatgt	agac	ccgtt	taagac	gcaagctaca	2160
caggaagtaa	aaaattgg	gacagaa	tttgtt	aaaatgc	tccaggat	2220
aagacttatt	taaaatgg	aggac	gtacact	aagaatgt	gacagcat	2280
caggatgtt	ggggaccc	ccataa	agat	ttttgg	ctgacat	2340
acaggttca	ctgc	ccataa	gtgc	ggcaat	gagccaa	2400
aagtgttca	attgtgg	agaagg	atagcc	gaaacaa	aaagactt	2460
aaggctgtt	ggaaatgtt	aaagg	agga	catcaat	aggatgc	2520
gctaattag						2529

<210> 17

<211> 3195

<212> DNA

<213> Artificial Sequence

<220>

<223> Hybrid protein cds comprised of Tat-Rev-Nef, CTL and truncated Gag protein (TRN-CTL-dgag)

<400> 17

atggagccag	tagatccat	actagagccc	tggaagcattc	caggaagtca	gcctaggacc	60
ccttgcacca	attgttattt	taaaaagtgt	tgccttcatt	gccaagttt	tttcacaaga	120
aaaggcttag	gcatctcta	tggcaggaa	aagcggagac	agcgacgaa	agctccctaa	180
gacagtca	ctcatca	ttcttacca	aagcaaccct	cctccagca	acgagggac	240
ccgacaggcc	cgaagaaatc	gaagaagaag	gtggagagag	agacagaggc	agatccgttc	300
gatactatgt	caggaagaag	cggagacagc	gacaaagagc	tcctcaagac	agtca	360
atcaagttt	tctaccaa	caaccctct	ccccagcaac	aggggacc	acaggcccga	420
agaaatcgaa	gaagaatgg	gagagagaga	cagaggcaga	tcggat	tagtgagcgg	480

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<210> 18
<211> 3195
<212> DNA
<213> Artificial Sequence
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<220>
<223> Hybrid protein cds comprised of Rev-Nef-Tat, CTL and truncated Gag
protein (RNT-CTL-dgag)

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<400> 18
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cgaagaagaa ggtggagaga gagacagagg catatccgtt cgatttagtgc gggatctt 180
agcacccccc tgggacgacc tgccggaccc ttgccttc agtgcaccggc gtttgagaga 240
cttacttgc attgttagcga aattgtggc aactctggc cgcgggggtt gggaaagtctt 300
caagatattq tggaaatctcc tgcgttattq qaqccaaqaa ctaaaagaaac tagtgtggc 360

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<210> 19
<211> 3195
<212> DNA
<213> Artificial Sequence

<220>
<223> Hybrid protein cds comprised of Tat-Rev-Nef, truncated Gag protein
and CTL (TRN-dgag-CTL)

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 gatactagt caggaagaag cggagacagc gaccaagagc tcctcaagac agtcagactc 360
 atcaagttc tctaccaaag caaccctcct cccagcaacg aggggaccgc acaggcccga 420
 agaaatcgaa gaagaaggta gagagagaga cagaggcaga tccgttgcgt tagtgacggg 480
 attcttaga ctttctggc acgacctgcg gacgcgtgc ctcttcagct accgcccgtt 540
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 agtcctcaag tattgggta atctctgcg tattgggac caggaactaa agaaaagctt 660
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<210> 20
 <211> 3195
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Hybrid protein cds comprised of Rev-Nef-Tat, truncated Gag protein and CTL (RNT-dgag-CTL)

<400> 20
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<210> 21
<211> 3020
<212> DNA
<213> Artificial Sequence
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<220>
<223> Hybrid protein cds comprised of Tat-Rev-Nef, truncated Gag protein
and CTL (TRN-optp17/24-CTL)

<400> 21

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tttgc	tttgc	tttgc	tttgc	tttgc	3020

<210> 22
<211> 3021

<212> DNA

<213> Artificial Sequence

<220>
<223> Hybrid protein cdscomprised of Tat-Rev-Nef, CTL and truncated Gag protein (TRN-CTL-optp17/24)

<400> 22
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<210> 23
<211> 3021
<212> DNA
<213> Artificial Sequence
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<220>
<223> Hybrid protein cds comprised of Rev-Nef-Tat, CTL and truncated Gag
protein (RNT-CTL-optp17/24)

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tttctctacc aaagcaaccc tcctccccgc aacqaaqqqda cccgacaccc cccgaagaataat 120

cgaagaagaa	ggtggagaga	gagacagagg	cagatccgtt	cgattagtga	gcggatttt	180
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cttactctt	attgttagga	agatgtgg	aactctgg	cgcagggggt	ggaaagtcc	300
caagtatttg	tggaatctcc	tgcgtattt	gagccaggaa	ctaaagaaac	tagtgtggc	360
aagtggtaa	aatgttagtg	atggctact	gtaaaggaaa	gaatgaaaca	agctgaggct	420
gagccagcag	cagatgggt	gggagcagca	tctcgagacc	tggaaaaca	tggagcaatc	480
acaagtagca	atacagaac	taataacgct	gtcttgcct	ggctagaagc	acaagaggaa	540
gaggaagtgg	gttttccact	cagacccct	gtacccctaa	gaccaatgac	ttacaaggga	600
gcttttagatc	ttagcccat	ttttaaagaa	aaggggggac	tggagggtt	aatttactcc	660
ccaaaaagac	aagatgctt	tgatctgtgg	gtcttaccaca	cacaaggcta	cttccctgtat	720
tggcagaact	acacaccagg	gccagggtc	agatatccac	tgacccttgg	atggtgcttc	780
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gcatttcata	acaaggccc	agagctgcat	ccggagact	acaaagactg	caagctttag	960
ccatgtatc	ctagactaga	gcccttggaa	catccaggaa	gtcagcttag	gacccttgt	1020
accaattgt	attgtaaaaa	gtgttgcctt	cattgccaag	tttggttcac	aagaaaaggc	1080
ttaggcatct	cctatggcag	gaagaagcgg	agacagcgc	gaagagctcc	tcaagacagt	1140
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gcccgtatca	ccctgtggca	gcgccttgc	gtggccctga	tcgagatctg	caccgagatg	1320
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<210> 24

<211> 3021

<212> DNA

<213> Artificial Sequence

<220>

<223> Hybrid protein cds comprised of Rev-Nef-Tat, truncated Gag protein and CTL (RNT-optp17/24-CTL)

<400> 24

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tttctctacc	aaagcaaccc	tcctcccagc	aacgagggga	cccgacaggc	ccgaagaaat	120
cgaagaagaa	ggtggagaga	gagacagagg	cagatccgtt	cgattagtga	gcggatttt	180

agcactttc tggacgacc tgcggagcct gtgccttcc agtaccgcc gcttgagaga 240
 cttaactctt attgttagcga agatttgtga aactctggg cgcagggggt gggagtctt 300
 caagtattgg tggaatctcc tgcagtattg gagccaggaa ctaaagaaac tagtgtggc 360
 aagtgtcaaa aatgttagtgg atggcctact gtaagggaaa gaatgaaaca agctgagcc 420
 gagccagcag catatgggtt gggagcagca ttcgcagacc tggaaaaaca tggagcaatc 480
 acaagttagca atacagcaac taataacgct gtttgcgtt ggctagaagc acaagaggaa 540
 gaggaaatggg gtttccagt cagacccatg gtacctttaa gaccaatgac ttacaaggga 600
 gcttttagatc ttagccactt tttaaaagaa aaggggggac tggagggtt aatttactcc 660
 ccaaaaagac aacagatctt tgatctgtgg gctctaccaca cacaaggctt cttccctgtat 720
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<210> 25

<211> 419

<212> PRT

<213> Artificial Sequence

<220>

<223> Hybrid protein comprised of Nef-Tat-Rev (NTR)

<400> 25

Met	Val	Gly	Lys	Trp	Ser	Lys	Cys	Ser	Gly	Trp	Pro	Thr	Val	Arg	Glu
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Arg	Met	Lys	Gln	Ala	Glu	Pro	Glu	Pro	Ala	Ala	Asp	Gly	Val	Gly	Ala
20															
Ala	Ser	Arg	Asp	Leu	Glu	Lys	His	Gly	Ala	Ile	Thr	Ser	Ser	Asn	Thr

35 40 45
 Ala Thr Asn Asn Ala Ala Cys Ala Trp Leu Glu Ala Gln Glu Glu Glu
 50 55 60
 Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr
 65 70 75 80
 Tyr Lys Gly Ala Leu Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly
 85 90 95
 Leu Glu Gly Leu Ile Tyr Ser Pro Lys Arg Gln Glu Ile Leu Asp Leu
 100 105 110
 Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr
 115 120 125
 Pro Gly Pro Gly Val Arg Tyr Pro Leu Thr Phe Gly Trp Cys Phe Lys
 130 135 140
 Leu Val Pro Val Glu Pro Asp Glu Glu Asn Ser Ser Leu Leu His
 145 150 155 160
 Pro Ala Ser Leu His Gly Thr Glu Asp Thr Glu Arg Glu Val Leu Lys
 165 170 175
 Trp Lys Phe Asp Ser His Leu Ala Phe His His Lys Ala Arg Glu Leu
 180 185 190
 His Pro Glu Tyr Tyr Lys Asp Cys Thr Ser Ala Gly Arg Ser Gly Asp
 195 200 205
 Ser Asp Glu Glu Leu Leu Lys Thr Val Arg Leu Ile Lys Phe Leu Tyr
 210 215 220
 Gln Ser Asn Pro Pro Ser Asn Glu Gly Thr Arg Gln Ala Arg Arg
 225 230 235 240
 Asn Arg Arg Arg Arg Trp Arg Glu Arg Gln Arg Gln Ile Arg Ser Ile
 245 250 255
 Ser Glu Arg Ile Leu Ser Thr Phe Leu Gly Arg Pro Ala Glu Pro Val
 260 265 270
 Pro Leu Gln Leu Pro Pro Leu Glu Arg Leu Thr Leu Asp Cys Ser Glu
 275 280 285
 Asp Cys Gly Asn Ser Gly Thr Gln Gly Val Gly Ser Pro Gln Val Leu
 290 295 300
 Val Glu Ser Pro Ala Val Leu Glu Pro Gly Thr Lys Glu Lys Leu Glu
 305 310 315 320
 Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser Gln Pro
 325 330 335
 Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Leu His Cys
 340 345 350
 Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly Arg Lys
 355 360 365
 Lys Arg Arg Gln Arg Arg Ala Pro Gln Asp Ser Gln Thr His Gln
 370 375 380
 Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Gln Arg Gly Asp Pro Thr
 385 390 395 400
 Gly Pro Lys Lys Ser Lys Lys Lys Val Glu Arg Glu Thr Glu Ala Asp
 405 410 415
 Pro Phe Asp

<210> 26

<211> 419

<212> PRT

<213> Artificial Sequence

<220>

<223> Hybrid protein comprised of Tat-Rev-Nef (TRN)

<400> 26

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 Gln Pro Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Leu
 20 25 30
 His Cys Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly

35 40 45
 Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro Gln Asp Ser Gln Thr
 50 55 60
 His Gln Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Gln Arg Gly Asp
 65 70 75 80
 Pro Thr Gly Pro Lys Lys Ser Lys Lys Lys Val Glu Arg Glu Thr Glu
 85 90 95
 Ala Asp Pro Phe Asp Thr Ser Ala Gly Arg Ser Gly Asp Ser Asp Glu
 100 105 110
 Glu Leu Leu Lys Thr Val Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn
 115 120 125
 Pro Pro Pro Ser Asn Glu Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg
 130 135 140
 Arg Arg Trp Arg Glu Arg Gln Ile Arg Ser Ile Ser Glu Arg
 145 150 155 160
 Ile Leu Ser Thr Phe Leu Gly Arg Pro Ala Glu Pro Val Pro Leu Gln
 165 170 175
 Leu Pro Pro Leu Glu Arg Leu Thr Leu Asp Cys Ser Glu Asp Cys Gly
 180 185 190
 Asn Ser Gly Thr Gln Gly Val Gly Ser Pro Gln Val Leu Val Glu Ser
 195 200 205
 Pro Ala Val Leu Glu Pro Gly Thr Lys Glu Lys Leu Val Gly Lys Trp
 210 215 220
 Ser Lys Cys Ser Gly Trp Pro Thr Val Arg Glu Arg Met Lys Gln Ala
 225 230 235 240
 Glu Pro Glu Pro Ala Ala Asp Gly Val Gly Ala Ala Ser Arg Asp Leu
 245 250 255
 Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala
 260 265 270
 Ala Cys Ala Trp Leu Glu Ala Gln Glu Glu Glu Val Gly Phe Pro
 275 280 285
 Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu
 290 295 300
 Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile
 305 310 315 320
 Tyr Ser Pro Lys Arg Gln Glu Ile Leu Asp Leu Trp Val Tyr His Thr
 325 330 335
 Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val
 340 345 350
 Arg Tyr Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu
 355 360 365
 Pro Asp Glu Glu Glu Asn Ser Ser Leu Leu His Pro Ala Ser Leu His
 370 375 380
 Gly Thr Glu Asp Thr Glu Arg Glu Val Leu Lys Trp Lys Phe Asp Ser
 385 390 395 400
 His Leu Ala Phe His His Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr
 405 410 415
 Lys Asp Cys

<210> 27

<211> 419

<212> PRT

<213> Artificial Sequence

<220>

<223> Hybrid protein comprised of Rev-Tat-Nef (RTN)

<400> 27

Met Ala Gly Arg Ser Gly Asp Ser Asp Glu Glu Leu Leu Lys Thr Val
 1 5 10 15
 Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn Pro Pro Pro Ser Asn Glu
 20 25 30
 Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg Arg Trp Arg Glu Arg

35 40 45
 Gln Arg Gln Ile Arg Ser Ile Ser Glu Arg Ile Leu Ser Thr Phe Leu
 50 55 60
 Gly Arg Pro Ala Glu Pro Val Pro Leu Gln Leu Pro Pro Leu Glu Arg
 65 70 75 80
 Leu Thr Leu Asp Cys Ser Glu Asp Cys Gly Asn Ser Gly Thr Gln Gly
 85 90 95
 Val Gly Ser Pro Gln Val Leu Val Glu Ser Pro Ala Val Leu Glu Pro
 100 105 110
 Gly Thr Lys Glu Thr Ser Glu Pro Val Asp Pro Arg Leu Glu Pro Trp
 115 120 125
 Lys His Pro Gly Ser Gln Pro Arg Thr Pro Cys Thr Asn Cys Tyr Cys
 130 135 140
 Lys Lys Cys Cys Leu His Cys Gln Val Cys Phe Thr Arg Lys Gly Leu
 145 150 155 160
 Gly Ile Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro
 165 170 175
 Gln Asp Ser Gln Thr His Gln Val Ser Leu Pro Lys Gln Pro Ser Ser
 180 185 190
 Gln Gln Arg Gly Asp Pro Thr Gly Pro Lys Lys Ser Lys Lys Lys Val
 195 200 205
 Glu Arg Glu Thr Glu Ala Asp Pro Phe Asp Lys Leu Val Gly Lys Trp
 210 215 220
 Ser Lys Cys Ser Gly Trp Pro Thr Val Arg Glu Arg Met Lys Gln Ala
 225 230 235 240
 Glu Pro Glu Pro Ala Ala Asp Gly Val Gly Ala Ala Ser Arg Asp Leu
 245 250 255
 Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala
 260 265 270
 Ala Cys Ala Trp Leu Glu Ala Gln Glu Glu Glu Val Gly Phe Pro
 275 280 285
 Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu
 290 295 300
 Asp Leu Ser His Phe Leu Lys Glu Lys Gly Leu Glu Gly Leu Ile
 305 310 315 320
 Tyr Ser Pro Lys Arg Gln Glu Ile Leu Asp Leu Trp Val Tyr His Thr
 325 330 335
 Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val
 340 345 350
 Arg Tyr Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu
 355 360 365
 Pro Asp Glu Glu Asn Ser Ser Leu Leu His Pro Ala Ser Leu His
 370 375 380
 Gly Thr Glu Asp Thr Glu Arg Glu Val Leu Lys Trp Lys Phe Asp Ser
 385 390 395 400
 His Leu Ala Phe His His Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr
 405 410 415
 Lys Asp Cys

<210> 28
 <211> 419
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Hybrid protein comprised of Tat-Nef-Rev (TNR)

<400> 28
 Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
 1 5 10 15
 Gln Pro Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Leu
 20 25 30
 His Cys Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly

35 40 45
 Arg Lys Lys Arg Arg Gln Arg Arg Ala Pro Gln Asp Ser Gln Thr
 50 55 60
 His Gln Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Gln Arg Gly Asp
 65 70 75 80
 Pro Thr Gly Pro Lys Lys Ser Lys Lys Val Glu Arg Glu Thr Glu
 85 90 95
 Ala Asp Pro Phe Asp Thr Ser Val Gly Lys Trp Ser Lys Cys Ser Gly
 100 105 110
 Trp Pro Thr Val Arg Glu Arg Met Lys Gln Ala Glu Pro Glu Pro Ala
 115 120 125
 Ala Asp Gly Val Gly Ala Ala Ser Arg Asp Leu Glu Lys His Gly Ala
 130 135 140
 Ile Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala Ala Cys Ala Trp Leu
 145 150 155 160
 Glu Ala Gln Glu Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val
 165 170 175
 Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu Asp Leu Ser His Phe
 180 185 190
 Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile Tyr Ser Pro Lys Arg
 195 200 205
 Gln Glu Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro
 210 215 220
 Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu Thr
 225 230 235 240
 Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Asp Glu Glu Glu
 245 250 255
 Asn Ser Ser Leu Leu His Pro Ala Ser Leu His Gly Thr Glu Asp Thr
 260 265 270
 Glu Arg Glu Val Leu Lys Trp Lys Phe Asp Ser His Leu Ala Phe His
 275 280 285
 His Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Lys Leu
 290 295 300
 Ala Gly Arg Ser Gly Asp Ser Asp Glu Glu Leu Leu Lys Thr Val Arg
 305 310 315 320
 Leu Ile Lys Phe Leu Tyr Gln Ser Asn Pro Pro Pro Ser Asn Glu Gly
 325 330 335
 Thr Arg Gln Ala Arg Arg Asn Arg Arg Arg Arg Trp Arg Glu Arg Gln
 340 345 350
 Arg Gln Ile Arg Ser Ile Ser Glu Arg Ile Leu Ser Thr Phe Leu Gly
 355 360 365
 Arg Pro Ala Glu Pro Val Pro Leu Gln Leu Pro Pro Leu Glu Arg Leu
 370 375 380
 Thr Leu Asp Cys Ser Glu Asp Cys Gly Asn Ser Gly Thr Gln Gly Val
 385 390 395 400
 Gly Ser Pro Gln Val Leu Val Glu Ser Pro Ala Val Leu Glu Pro Gly
 405 410 415
 Thr Lys Glu

<210> 29
 <211> 419
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Hybrid protein comprised of Rev-Nef-Tat (RNT)

<400> 29
 Met Ala Gly Arg Ser Gly Asp Ser Asp Glu Glu Leu Leu Lys Thr Val
 1 5 10 15
 Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn Pro Pro Pro Ser Asn Glu
 20 25 30
 Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg Arg Trp Arg Glu Arg

35 40 45
 Gln Arg Gln Ile Arg Ser Ile Ser Glu Arg Ile Leu Ser Thr Phe Leu
 50 55 60
 Gly Arg Pro Ala Glu Pro Val Pro Leu Gln Leu Pro Pro Leu Glu Arg
 65 70 75 80
 Leu Thr Leu Asp Cys Ser Glu Asp Cys Gly Asn Ser Gly Thr Gln Gly
 85 90 95
 Val Gly Ser Pro Gln Val Leu Val Glu Ser Pro Ala Val Leu Glu Pro
 100 105 110
 Gly Thr Lys Glu Thr Ser Val Gly Lys Trp Ser Lys Cys Ser Gly Trp
 115 120 125
 Pro Thr Val Arg Glu Arg Met Lys Gln Ala Glu Pro Glu Pro Ala Ala
 130 135 140
 Asp Gly Val Gly Ala Ala Ser Arg Asp Leu Glu Lys His Gly Ala Ile
 145 150 155 160
 Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala Ala Cys Ala Trp Leu Glu
 165 170 175
 Ala Gln Glu Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro
 180 185 190
 Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu Asp Leu Ser His Phe Leu
 195 200 205
 Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile Tyr Ser Pro Lys Arg Gln
 210 215 220
 Glu Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp
 225 230 235 240
 Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu Thr Phe
 245 250 255
 Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Asp Glu Glu Asn
 260 265 270
 Ser Ser Leu Leu His Pro Ala Ser Leu His Gly Thr Glu Asp Thr Glu
 275 280 285
 Arg Glu Val Leu Lys Trp Lys Phe Asp Ser His Leu Ala Phe His His
 290 295 300
 Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Lys Leu Glu
 305 310 315 320
 Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser Gln Pro
 325 330 335
 Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Leu His Cys
 340 345 350
 Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly Arg Lys
 355 360 365
 Lys Arg Arg Gln Arg Arg Ala Pro Gln Asp Ser Gln Thr His Gln
 370 375 380
 Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Gln Arg Gly Asp Pro Thr
 385 390 395 400
 Gly Pro Lys Lys Ser Lys Lys Lys Val Glu Arg Glu Thr Glu Ala Asp
 405 410 415
 Pro Phe Asp

<210> 30

<211> 387

<212> PRT

<213> Artificial Sequence

<220> Protein comprised of Immunodominant parts of the Nef-Tat-Rev(NTR)

<400> 30
 Met Gly Trp Pro Thr Val Arg Glu Arg Met Lys Gln Ala Glu Pro Glu
 5 10 15
 Pro Ala Ala Asp Gly Val Gly Ala Ala Ser Arg Asp Leu Glu Lys His
 20 25 30
 Gly Ala Ile Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala Ala Cys Ala

35 40 45
 Trp Leu Glu Ala Gln Glu Glu Glu Val Gly Phe Pro Val Arg Pro
 50 55 60
 Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu Asp Leu Ser
 65 70 75 80
 His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile Tyr Ser Pro
 85 90 95
 Lys Arg Gln Glu Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr
 100 105 110
 Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro
 115 120 125
 Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Asp Glu
 130 135 140
 Glu Glu Asn Ser Ser Leu Leu His Pro Ala Ser Leu His Gly Thr Glu
 145 150 155 160
 Asp Thr Glu Arg Glu Val Leu Lys Trp Lys Phe Asp Ser His Leu Ala
 165 170 175
 Phe His His Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys
 180 185 190
 Ala Leu Ala Ala Val Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys
 195 200 205
 His Pro Gly Ser Gln Pro Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys
 210 215 220
 Lys Cys Cys Leu His Cys Gln Val Cys Phe Thr Arg Lys Gly Leu Gly
 225 230 235 240
 Ile Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro Gln
 245 250 255
 Asp Ser Gln Thr His Gln Val Ser Leu Pro Lys Gln Pro Ser Ser Gln
 260 265 270
 Gln Arg Gly Asp Pro Thr Gly Pro Lys Lys Ser Gly Leu Ala Ile Leu
 275 280 285
 Leu Ser Asp Glu Glu Leu Leu Lys Thr Val Arg Leu Ile Lys Phe Leu
 290 295 300
 Tyr Gln Ser Asn Pro Pro Pro Ser Asn Glu Gly Thr Arg Gln Ala Arg
 305 310 315 320
 Arg Asn Arg Arg Arg Trp Arg Glu Arg Gln Arg Gln Ile Arg Ser
 325 330 335
 Ile Ser Glu Arg Ile Leu Ser Thr Phe Leu Gly Arg Pro Ala Glu Pro
 340 345 350
 Val Pro Leu Gln Leu Pro Pro Leu Glu Arg Leu Thr Leu Asp Cys Ser
 355 360 365
 Glu Asp Cys Gly Asn Ser Gly Thr Gln Gly Val Gly Ser Pro Gln Val
 370 375 380
 Leu Val Glu
 385

<210> 31
 <211> 390
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Protein comprised of Immunodominant parts of the Nef-Tat-Rev separated by protease sites(NTR)

<400> 31
 Met Gly Trp Pro Thr Val Arg Glu Arg Met Lys Gln Ala Glu Pro Glu
 1 5 10 15
 Pro Ala Ala Asp Gly Val Gly Ala Ala Ser Arg Asp Leu Glu Lys His
 20 25 30
 Gly Ala Ile Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala Ala Cys Ala
 35 40 45
 Trp Leu Glu Ala Gln Glu Glu Glu Val Gly Phe Pro Val Arg Pro

50 55 60
 Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu Asp Leu Ser
 65 70 75 80
 His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile Tyr Ser Pro
 85 90 95
 Lys Arg Gln Glu Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr
 100 105 110
 Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro
 115 120 125
 Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Asp Glu
 130 135 140
 Glu Glu Asn Ser Ser Leu Leu His Pro Ala Ser Leu His Gly Thr Glu
 145 150 155 160
 Asp Thr Glu Arg Glu Val Leu Lys Trp Lys Phe Asp Ser His Leu Ala
 165 170 175
 Phe His His Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys
 180 185 190
 Ala Leu Ala Phe Lys Arg Val Glu Pro Val Asp Pro Arg Leu Glu Pro
 195 200 205
 Trp Lys His Pro Gly Ser Gln Pro Arg Thr Pro Cys Thr Asn Cys Tyr
 210 215 220
 Cys Lys Lys Cys Cys Leu His Cys Gln Val Cys Phe Thr Arg Lys Gly
 225 230 235 240
 Leu Gly Ile Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala
 245 250 255
 Pro Gln Asp Ser Gln Thr His Gln Val Ser Leu Pro Lys Gln Pro Ser
 260 265 270
 Ser Gln Gln Arg Gly Asp Pro Thr Gly Pro Lys Lys Ser Val Arg Glu
 275 280 285
 Lys Arg Leu Leu Ser Asp Glu Glu Leu Leu Lys Thr Val Arg Leu Ile
 290 295 300
 Lys Phe Leu Tyr Gln Ser Asn Pro Pro Ser Asn Glu Gly Thr Arg
 305 310 315 320
 Gln Ala Arg Arg Asn Arg Arg Arg Arg Trp Arg Glu Arg Gln Arg Gln
 325 330 335
 Ile Arg Ser Ile Ser Glu Arg Ile Leu Ser Thr Phe Leu Gly Arg Pro
 340 345 350
 Ala Glu Pro Val Pro Leu Gln Leu Pro Pro Leu Glu Arg Leu Thr Leu
 355 360 365
 Asp Cys Ser Glu Asp Cys Gly Asn Ser Gly Thr Gln Gly Val Gly Ser
 370 375 380
 Pro Gln Val Leu Val Glu
 385 390

<210> 32

<211> 386

<212> PRT

<213> Artificial Sequence

<220>

<223> Protein comprised of Immunodominant parts of the regulatory proteins
Nef-Tat-Rev started from aal of Nef(N11TR)

<400> 32

Met Trp Pro Thr Val Arg Glu Arg Met Lys Gln Ala Glu Pro Glu Pro
 1 5 10 15
 Ala Ala Asp Gly Val Gly Ala Ala Ser Arg Asp Leu Glu Lys His Gly
 20 25 30
 Ala Ile Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala Ala Cys Ala Trp
 35 40 45
 Leu Glu Ala Gln Glu Glu Glu Val Gly Phe Pro Val Arg Pro Gln
 50 55 60
 Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu Asp Leu Ser His

65 70 75 80
 Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile Tyr Ser Pro Lys
 85 90 95
 Arg Gln Glu Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe
 100 105 110
 Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu
 115 120 125
 Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Asp Glu Glu
 130 135 140
 Glu Asn Ser Ser Leu Leu His Pro Ala Ser Leu His Gly Thr Glu Asp
 145 150 155 160
 Thr Glu Arg Glu Val Leu Lys Trp Lys Phe Asp Ser His Leu Ala Phe
 165 170 175
 His His Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Ala
 180 185 190
 Leu Ala Ala Val Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His
 195 200 205
 Pro Gly Ser Gln Pro Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys
 210 215 220
 Cys Cys Leu His Cys Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile
 225 230 235 240
 Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro Gln Asp
 245 250 255
 Ser Gln Thr His Gln Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Gln
 260 265 270
 Arg Gly Asp Pro Thr Gly Pro Lys Ser Gly Leu Ala Ile Leu Leu
 275 280 285
 Ser Asp Glu Glu Leu Leu Lys Thr Val Arg Leu Ile Lys Phe Leu Tyr
 290 295 300
 Gln Ser Asn Pro Pro Pro Ser Asn Glu Gly Thr Arg Gln Ala Arg Arg
 305 310 315 320
 Asn Arg Arg Arg Trp Arg Glu Arg Gln Arg Gln Ile Arg Ser Ile
 325 330 335
 Ser Glu Arg Ile Leu Ser Thr Phe Leu Gly Arg Pro Ala Glu Pro Val
 340 345 350
 Pro Leu Gln Leu Pro Pro Leu Glu Arg Leu Thr Leu Asp Cys Ser Glu
 355 360 365
 Asp Cys Gly Asn Ser Gly Thr Gln Gly Val Gly Ser Pro Gln Val Leu
 370 375 380
 Val Glu
 385

<210> 33
 <211> 389
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Protein comprised of Immunodominant parts of the regulatory proteins
 Nef-Tat-Rev started from aal of Nef separated by protease sites(N11TR)

<400> 33
 Met Trp Pro Thr Val Arg Glu Arg Met Lys Gln Ala Glu Pro Glu Pro
 1 5 10 15
 Ala Ala Asp Gly Val Gly Ala Ala Ser Arg Asp Leu Glu Lys His Gly
 20 25 30
 Ala Ile Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala Ala Cys Ala Trp
 35 40 45
 Leu Glu Ala Gln Glu Glu Glu Val Gly Phe Pro Val Arg Pro Gln
 50 55 60
 Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu Asp Leu Ser His
 65 70 75 80
 Phe Leu Lys Glu Lys Gly Leu Glu Gly Leu Ile Tyr Ser Pro Lys

85 90 95
 Arg Gln Glu Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe
 100 105 110
 Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu
 115 120 125
 Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Asp Glu Glu
 130 135 140
 Glu Asn Ser Ser Leu Leu His Pro Ala Ser Leu His Gly Thr Glu Asp
 145 150 155 160
 Thr Glu Arg Glu Val Leu Lys Trp Lys Phe Asp Ser His Leu Ala Phe
 165 170 175
 His His Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Ala
 180 185 190
 Leu Ala Phe Lys Arg Val Glu Pro Val Asp Pro Arg Leu Glu Pro Trp
 195 200 205
 Lys His Pro Gly Ser Gln Pro Arg Thr Pro Cys Thr Asn Cys Tyr Cys
 210 215 220
 Lys Lys Cys Cys Leu His Cys Gln Val Cys Phe Thr Arg Lys Gly Leu
 225 230 235 240
 Gly Ile Ser Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro
 245 250 255
 Gln Asp Ser Gln Thr His Gln Val Ser Leu Pro Lys Gln Pro Ser Ser
 260 265 270
 Gln Gln Arg Gly Asp Pro Thr Gly Pro Lys Lys Ser Val Arg Glu Lys
 275 280 285
 Arg Leu Leu Ser Asp Glu Glu Leu Leu Lys Thr Val Arg Leu Ile Lys
 290 295 300
 Phe Leu Tyr Gln Ser Asn Pro Pro Ser Asn Glu Gly Thr Arg Gln
 305 310 315 320
 Ala Arg Arg Asn Arg Arg Arg Arg Trp Arg Glu Arg Gln Arg Gln Ile
 325 330 335
 Arg Ser Ile Ser Glu Arg Ile Leu Ser Thr Phe Leu Gly Arg Pro Ala
 340 345 350
 Glu Pro Val Pro Leu Gln Leu Pro Pro Leu Glu Arg Leu Thr Leu Asp
 355 360 365
 Cys Ser Glu Asp Cys Gly Asn Ser Gly Thr Gln Gly Val Gly Ser Pro
 370 375 380
 Gln Val Leu Val Glu
 385

<210> 34
 <211> 220
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Protein comprised of Cytotoxic T-cell epitopes of Pol and Env genes(CTL)

<400> 34
 Met Ile Thr Leu Trp Gln Arg Pro Leu Val Ala Leu Ile Glu Ile Cys
 1 5 10 15
 Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Ala Gly
 20 25 30
 Leu Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr
 35 40 45
 Phe Ser Val Pro Leu Asp Lys Asp Phe Arg Lys Tyr Thr Ala Phe Thr
 50 55 60
 Ile Pro Ser Ile Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met
 65 70 75 80
 Thr Lys Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp
 85 90 95
 Leu Tyr Val Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Leu Val Gly

100 105 110
 Lys Leu Asn Trp Ala Ser Gln Ile Tyr Ala Gly Ile Lys Val Lys Gln
 115 120 125
 Leu Ile Leu Lys Glu Pro Val His Gly Val Tyr Glu Pro Ile Val Gly
 130 135 140
 Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Ala Gly Asn Leu
 145 150 155 160
 Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Thr Thr
 165 170 175
 Thr Leu Val Glu Arg Tyr Leu Arg Asp Gln Gln Leu Leu Gly Ile Trp
 180 185 190
 Gly Cys Ala Cys Thr Pro Tyr Asp Ile Asn Gln Met Leu Arg Gly Pro
 195 200 205
 Gly Arg Ala Phe Val Thr Ile Arg Gln Gly Ser Leu
 210 215 220

<210> 35

<211> 421

<212> PRT

<213> Artificial Sequence

<220>

<223> Truncated Gag protein sequence(dgag)

<400> 35

Met Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys
 1 5 10 15
 Lys Tyr Gln Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg
 20 25 30
 Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln
 35 40 45
 Ile Met Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu
 50 55 60
 Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys
 65 70 75 80
 Ile Glu Val Lys Asp Thr Lys Glu Ala Leu Asp Lys Val Glu Glu Glu
 85 90 95
 Gln Asn Asn Ser Lys Lys Lys Ala Gln Gln Glu Ala Ala Asp Ala Gly
 100 105 110
 Asn Arg Asn Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln
 115 120 125
 Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp
 130 135 140
 Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met
 145 150 155 160
 Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met
 165 170 175
 Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu
 180 185 190
 Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His
 195 200 205
 Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp
 210 215 220
 Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr
 225 230 235 240
 Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile
 245 250 255
 Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu
 260 265 270
 Asp Ile Lys Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg
 275 280 285
 Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Thr Gln Glu Val Lys Asn
 290 295 300

Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys
 305 310 315 320
 Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met
 325 330 335
 Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu
 340 345 350
 Ala Glu Ala Met Ser Gln Val Thr Gly Ser Ala Ala Ile Met Met Gln
 355 360 365
 Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys
 370 375 380
 Gly Lys Glu Gly His Ile Ala Arg Asn Cys Arg Ala Pro Arg Lys Lys
 385 390 395 400
 Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Thr
 405 410 415
 Glu Arg Gln Ala Asn
 420

<210> 36
 <211> 363
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic p17/24 protein of Gag gene (Syn 17/24)

<400> 36
 Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp
 1 5 10 15
 Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Gln Leu Lys
 20 25 30
 His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro
 35 40 45
 Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Met Gly Gln Leu
 50 55 60
 Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn
 65 70 75 80
 Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Glu Val Lys Asp
 85 90 95
 Thr Lys Glu Ala Leu Asp Lys Val Glu Glu Glu Gln Asn Asn Ser Lys
 100 105 110
 Lys Lys Ala Gln Gln Glu Ala Ala Asp Ala Gly Asn Arg Asn Gln Val
 115 120 125
 Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His
 130 135 140
 Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Glu
 145 150 155 160
 Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser
 165 170 175
 Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly
 180 185 190
 Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu
 195 200 205
 Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala
 210 215 220
 Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr
 225 230 235 240
 Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile
 245 250 255
 Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys
 260 265 270
 Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Lys Gln Gly
 275 280 285
 Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu

290 295 300
 Arg Ala Glu Gln Ala Thr Gln Glu Val Lys Asn Trp Met Thr Glu Thr
 305 310 315 320
 Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala
 325 330 335
 Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly
 340 345 350
 Val Gly Gly Pro Gly His Lys Ala Arg Val Leu
 355 360

<210> 37
 <211> 363
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic p17/24 protein of Gag gene optimized for expression in
 eukaryotic cells(optp 17/24)

<400> 37
 Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp
 1 5 10 15
 Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Tyr Gln Leu Lys
 20 25 30
 His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro
 35 40 45
 Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Met Gly Gln Leu
 50 55 60
 Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn
 65 70 75 80
 Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Glu Val Lys Asp
 85 90 95
 Thr Lys Glu Ala Leu Asp Lys Val Glu Glu Glu Gln Asn Asn Ser Lys
 100 105 110
 Lys Lys Ala Gln Gln Glu Ala Ala Asp Ala Gly Asn Arg Asn Gln Val
 115 120 125
 Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His
 130 135 140
 Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu
 145 150 155 160
 Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser
 165 170 175
 Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly
 180 185 190
 Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu
 195 200 205
 Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala
 210 215 220
 Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr
 225 230 235 240
 Ser Thr Leu Gln Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile
 245 250 255
 Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys
 260 265 270
 Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Lys Gln Gly
 275 280 285
 Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu
 290 295 300
 Arg Ala Glu Gln Ala Thr Gln Glu Val Lys Asn Trp Met Thr Glu Thr
 305 310 315 320
 Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala
 325 330 335
 Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly

340 345 350

Val Gly Gly Pro Gly His Lys Ala Arg Val Leu
 355 360

<210> 38
<211> 641
<212> PRT
<213> Artificial Sequence

<220>
<223> Hybrid protein comprised of Tat-Rev-Nef and CTL(TRN-CTL)

<400> 38
Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
 1 5 10 15
Gln Pro Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Leu
 20 25 30
His Cys Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly
 35 40 45
Arg Lys Lys Arg Arg Gln Arg Arg Ala Pro Gln Asp Ser Gln Thr
 50 55 60
His Gln Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Arg Gly Asp
 65 70 75 80
Pro Thr Gly Pro Lys Lys Ser Lys Lys Val Glu Arg Glu Thr Glu
 85 90 95
Ala Asp Pro Phe Asp Thr Ser Ala Gly Arg Ser Gly Asp Ser Asp Glu
 100 105 110
Glu Leu Leu Lys Thr Val Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn
 115 120 125
Pro Pro Pro Ser Asn Glu Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg
 130 135 140
Arg Arg Trp Arg Glu Arg Gln Arg Ile Arg Ser Ile Ser Glu Arg
 145 150 155 160
Ile Leu Ser Thr Phe Leu Gly Arg Pro Ala Glu Pro Val Pro Leu Gln
 165 170 175
Leu Pro Pro Leu Glu Arg Leu Thr Leu Asp Cys Ser Glu Asp Cys Gly
 180 185 190
Asn Ser Gly Thr Gln Gly Val Gly Ser Pro Gln Val Leu Val Glu Ser
 195 200 205
Pro Ala Val Leu Glu Pro Gly Thr Lys Glu Lys Leu Val Gly Lys Trp
 210 215 220
Ser Lys Cys Ser Gly Trp Pro Thr Val Arg Glu Arg Met Lys Gln Ala
 225 230 235 240
Glu Pro Glu Pro Ala Ala Asp Gly Val Gly Ala Ala Ser Arg Asp Leu
 245 250 255
Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala
 260 265 270
Ala Cys Ala Trp Leu Glu Ala Gln Glu Glu Glu Val Gly Phe Pro
 275 280 285
Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu
 290 295 300
Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile
 305 310 315 320
Tyr Ser Pro Lys Arg Gln Glu Ile Leu Asp Leu Trp Val Tyr His Thr
 325 330 335
Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val
 340 345 350
Arg Tyr Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu
 355 360 365
Pro Asp Glu Glu Asn Ser Ser Leu Leu His Pro Ala Ser Leu His
 370 375 380
Gly Thr Glu Asp Thr Glu Arg Glu Val Leu Lys Trp Lys Phe Asp Ser
 385 390 395 400

His Leu Ala Phe His His Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr
 405 410 415
 Lys Asp Cys Ala Ala Val Ile Thr Leu Trp Gln Arg Pro Leu Val Ala
 420 425 430
 Leu Ile Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys
 435 440 445
 Ile Gly Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp
 450 455 460
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Lys Asp Phe Arg Lys
 465 470 475 480
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Trp Lys Gly Ser Pro Ala Ile
 485 490 495
 Phe Gln Ser Ser Met Thr Lys Lys Gln Asn Pro Asp Ile Val Ile Tyr
 500 505 510
 Gln Tyr Met Asp Asp Leu Tyr Val Pro Ile Val Leu Pro Glu Lys Asp
 515 520 525
 Ser Trp Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Ala Gly
 530 535 540
 Ile Lys Val Lys Gln Leu Ile Leu Lys Glu Pro Val His Gly Val Tyr
 545 550 555 560
 Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn
 565 570 575
 Arg Ala Gly Asn Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp
 580 585 590
 Lys Glu Ala Thr Thr Thr Leu Val Glu Arg Tyr Leu Arg Asp Gln Gln
 595 600 605
 Leu Leu Gly Ile Trp Gly Cys Ala Cys Thr Pro Tyr Asp Ile Asn Gln
 610 615 620
 Met Leu Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Arg Gln Gly Ser
 625 630 635 640
 Leu

<210> 39
 <211> 641
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Hybrid protein comprised of Rev-Nef-Tat and CTL(RNT-CTL)

<400> 39
 Met Ala Gly Arg Ser Gly Asp Ser Asp Glu Glu Leu Leu Lys Thr Val
 1 5 10 15
 Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn Pro Pro Pro Ser Asn Glu
 20 25 30
 Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg Arg Arg Trp Arg Glu Arg
 35 40 45
 Gln Arg Gln Ile Arg Ser Ile Ser Glu Arg Ile Leu Ser Thr Phe Leu
 50 55 60
 Gly Arg Pro Ala Glu Pro Val Pro Leu Gln Leu Pro Pro Leu Glu Arg
 65 70 75 80
 Leu Thr Leu Asp Cys Ser Glu Asp Cys Gly Asn Ser Gly Thr Gln Gly
 85 90 95
 Val Gly Ser Pro Gln Val Leu Val Glu Ser Pro Ala Val Leu Glu Pro
 100 105 110
 Gly Thr Lys Glu Thr Ser Val Gly Lys Trp Ser Lys Cys Ser Gly Trp
 115 120 125
 Pro Thr Val Arg Glu Arg Met Lys Gln Ala Glu Pro Glu Pro Ala Ala
 130 135 140
 Asp Gly Val Gly Ala Ala Ser Arg Asp Leu Glu Lys His Gly Ala Ile
 145 150 155 160
 Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala Cys Ala Trp Leu Glu

165 170 175
 Ala Gln Glu Glu Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro
 180 185 190
 Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu Asp Leu Ser His Phe Leu
 195 200 205
 Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile Tyr Ser Pro Lys Arg Gln
 210 215 220
 Glu Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp
 225 230 235 240
 Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu Thr Phe
 245 250 255
 Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Asp Glu Glu Asn
 260 265 270
 Ser Ser Leu Leu His Pro Ala Ser Leu His Gly Thr Glu Asp Thr Glu
 275 280 285
 Arg Glu Val Leu Lys Trp Lys Phe Asp Ser His Leu Ala Phe His His
 290 295 300
 Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Lys Leu Glu
 305 310 315 320
 Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser Gln Pro
 325 330 335
 Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Leu His Cys
 340 345 350
 Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly Arg Lys
 355 360 365
 Lys Arg Arg Gln Arg Arg Arg Ala Pro Gln Asp Ser Gln Thr His Gln
 370 375 380
 Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Gln Arg Gly Asp Pro Thr
 385 390 395 400
 Gly Pro Lys Lys Ser Lys Lys Val Glu Arg Glu Thr Glu Ala Asp
 405 410 415
 Pro Phe Asp Ala Ala Val Ile Thr Leu Trp Gln Arg Pro Leu Val Ala
 420 425 430
 Leu Ile Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys
 435 440 445
 Ile Gly Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp
 450 455 460
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Lys Asp Phe Arg Lys
 465 470 475 480
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Trp Lys Gly Ser Pro Ala Ile
 485 490 495
 Phe Gln Ser Ser Met Thr Lys Lys Gln Asn Pro Asp Ile Val Ile Tyr
 500 505 510
 Gln Tyr Met Asp Asp Leu Tyr Val Pro Ile Val Leu Pro Glu Lys Asp
 515 520 525
 Ser Trp Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Ala Gly
 530 535 540
 Ile Lys Val Lys Gln Leu Ile Leu Lys Glu Pro Val His Gly Val Tyr
 545 550 555 560
 Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn
 565 570 575
 Arg Ala Gly Asn Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp
 580 585 590
 Lys Glu Ala Thr Thr Thr Leu Val Glu Arg Tyr Leu Arg Asp Gln Gln
 595 600 605
 Leu Leu Gly Ile Trp Gly Cys Ala Cys Thr Pro Tyr Asp Ile Asn Gln
 610 615 620
 Met Leu Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Arg Gln Gly Ser
 625 630 635 640
 Leu

<210> 40
 <211> 842

<212> PRT
 <213> Artificial Sequence

<220>
 <223> Hybrid protein cds comprised of Tat-Rev-Nef and truncated Gag protein(TRN-dgag)

<400> 40
 Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
 1 5 10 15
 Gln Pro Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Leu
 20 25 30
 His Cys Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly
 35 40 45
 Arg Lys Lys Arg Arg Gln Arg Arg Ala Pro Gln Asp Ser Gln Thr
 50 55 60
 His Gln Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Arg Gly Asp
 65 70 75 80
 Pro Thr Gly Pro Lys Lys Ser Lys Lys Val Glu Arg Glu Thr Glu
 85 90 95
 Ala Asp Pro Phe Asp Thr Ser Ala Gly Arg Ser Gly Asp Ser Asp Glu
 100 105 110
 Glu Leu Leu Lys Thr Val Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn
 115 120 125
 Pro Pro Pro Ser Asn Glu Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg
 130 135 140
 Arg Arg Trp Arg Glu Arg Gln Arg Gln Ile Arg Ser Ile Ser Glu Arg
 145 150 155 160
 Ile Leu Ser Thr Phe Leu Gly Arg Pro Ala Glu Pro Val Pro Leu Gln
 165 170 175
 Leu Pro Pro Leu Glu Arg Leu Thr Leu Asp Cys Ser Glu Asp Cys Gly
 180 185 190
 Asn Ser Gly Thr Gln Gly Val Gly Ser Pro Gln Val Leu Val Glu Ser
 195 200 205
 Pro Ala Val Leu Glu Pro Gly Thr Lys Glu Lys Leu Val Gly Lys Trp
 210 215 220
 Ser Lys Cys Ser Gly Trp Pro Thr Val Arg Glu Arg Met Lys Gln Ala
 225 230 235 240
 Glu Pro Glu Pro Ala Ala Asp Gly Val Gly Ala Ala Ser Arg Asp Leu
 245 250 255
 Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala
 260 265 270
 Ala Cys Ala Trp Leu Glu Ala Gln Glu Glu Glu Val Gly Phe Pro
 275 280 285
 Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu
 290 295 300
 Asp Leu Ser His Phe Leu Lys Glu Lys Gly Leu Glu Gly Leu Ile
 305 310 315 320
 Tyr Ser Pro Lys Arg Gln Glu Ile Leu Asp Leu Trp Val Tyr His Thr
 325 330 335
 Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val
 340 345 350
 Arg Tyr Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu
 355 360 365
 Pro Asp Glu Glu Asn Ser Ser Leu Leu His Pro Ala Ser Leu His
 370 375 380
 Gly Thr Glu Asp Thr Glu Arg Glu Val Leu Lys Trp Lys Phe Asp Ser
 385 390 395 400
 His Leu Ala Phe His His Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr
 405 410 415
 Lys Asp Cys Ala Ala Val Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg
 420 425 430
 Pro Gly Gly Lys Lys Tyr Gln Leu Lys His Ile Val Trp Ala Ser

435 440 445
 Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser
 450 455 460
 Glu Gly Cys Arg Gln Ile Met Gly Gln Leu Gln Pro Ser Leu Gln Thr
 465 470 475 480
 Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr
 485 490 495
 Cys Val His Gln Lys Ile Glu Val Lys Asp Thr Lys Glu Ala Leu Asp
 500 505 510
 Lys Val Glu Glu Gln Asn Asn Ser Lys Lys Lys Ala Gln Gln Glu
 515 520 525
 Ala Ala Asp Ala Gly Asn Arg Asn Gln Val Ser Gln Asn Tyr Pro Ile
 530 535 540
 Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg
 545 550 555 560
 Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro
 565 570 575
 Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln
 580 585 590
 Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met
 595 600 605
 Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg
 610 615 620
 Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu
 625 630 635 640
 Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln
 645 650 655
 Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr
 660 665 670
 Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser
 675 680 685
 Pro Thr Ser Ile Leu Asp Ile Lys Gln Gly Pro Lys Glu Pro Phe Arg
 690 695 700
 Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Thr
 705 710 715 720
 Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala
 725 730 735
 Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr
 740 745 750
 Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His
 755 760 765
 Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Gly Ser Ala
 770 775 780
 Ala Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val
 785 790 795 800
 Lys Cys Phe Asn Cys Gly Lys Glu Gly His Ile Ala Arg Asn Cys Arg
 805 810 815
 Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln
 820 825 830
 Met Lys Asp Cys Thr Glu Arg Gln Ala Asn
 835 840

<210> 41
 <211> 1064
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Hybrid protein cds comprised of Tat-Rev-Nef, CTL and truncated Gag
 protein(TRN-TCL-dgag)

<400> 41
 Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser

1 5 10 15
 Gln Pro Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Leu
 20 25 30
 His Cys Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly
 35 40 45
 Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro Gln Asp Ser Gln Thr
 50 55 60
 His Gln Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Gln Arg Gly Asp
 65 70 75 80
 Pro Thr Gly Pro Lys Lys Ser Lys Lys Val Glu Arg Glu Thr Glu
 85 90 95
 Ala Asp Pro Phe Asp Thr Ser Ala Gly Arg Ser Gly Asp Ser Asp Glu
 100 105 110
 Glu Leu Leu Lys Thr Val Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn
 115 120 125
 Pro Pro Pro Ser Asn Glu Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg
 130 135 140
 Arg Arg Trp Arg Glu Arg Gln Arg Gln Ile Arg Ser Ile Ser Glu Arg
 145 150 155 160
 Ile Leu Ser Thr Phe Leu Gly Arg Pro Ala Glu Pro Val Pro Leu Gln
 165 170 175
 Leu Pro Pro Leu Glu Arg Leu Thr Leu Asp Cys Ser Glu Asp Cys Gly
 180 185 190
 Asn Ser Gly Thr Gln Gly Val Gly Ser Pro Gln Val Leu Val Glu Ser
 195 200 205
 Pro Ala Val Leu Glu Pro Gly Thr Lys Glu Lys Leu Val Gly Lys Trp
 210 215 220
 Ser Lys Cys Ser Gly Trp Pro Thr Val Arg Glu Arg Met Lys Gln Ala
 225 230 235 240
 Glu Pro Glu Pro Ala Ala Asp Gly Val Gly Ala Ala Ser Arg Asp Leu
 245 250 255
 Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala
 260 265 270
 Ala Cys Ala Trp Leu Glu Ala Gln Glu Glu Glu Val Gly Phe Pro
 275 280 285
 Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu
 290 295 300
 Asp Leu Ser His Phe Leu Lys Glu Lys Gly Leu Glu Gly Leu Ile
 305 310 315 320
 Tyr Ser Pro Lys Arg Gln Glu Ile Leu Asp Leu Trp Val Tyr His Thr
 325 330 335
 Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val
 340 345 350
 Arg Tyr Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu
 355 360 365
 Pro Asp Glu Glu Asn Ser Ser Leu Leu His Pro Ala Ser Leu His
 370 375 380
 Gly Thr Glu Asp Thr Glu Arg Glu Val Leu Lys Trp Lys Phe Asp Ser
 385 390 395 400
 His Leu Ala Phe His His Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr
 405 410 415
 Lys Asp Cys Ala Ala Val Ile Thr Leu Trp Gln Arg Pro Leu Val Ala
 420 425 430
 Leu Ile Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys
 435 440 445
 Ile Gly Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp
 450 455 460
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Lys Asp Phe Arg Lys
 465 470 475 480
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Trp Lys Gly Ser Pro Ala Ile
 485 490 495
 Phe Gln Ser Ser Met Thr Lys Lys Gln Asn Pro Asp Ile Val Ile Tyr
 500 505 510

Gln Tyr Met Asp Asp Leu Tyr Val Pro Ile Val Leu Pro Glu Lys Asp
 515 520 525
 Ser Trp Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Ala Gly
 530 535 540
 Ile Lys Val Lys Gln Leu Ile Leu Lys Glu Pro Val His Gly Val Tyr
 545 550 555 560
 Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn
 565 570 575
 Arg Ala Gly Asn Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp
 580 585 590
 Lys Glu Ala Thr Thr Thr Leu Val Glu Arg Tyr Leu Arg Asp Gln Gln
 595 600 605
 Leu Leu Gly Ile Trp Gly Cys Ala Cys Thr Pro Tyr Asp Ile Asn Gln
 610 615 620
 Met Leu Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Arg Gln Gly Ser
 625 630 635 640
 Leu Ala Ala Val Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly
 645 650 655
 Gly Lys Lys Tyr Gln Leu Lys His Ile Val Trp Ala Ser Arg Glu
 660 665 670
 Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly
 675 680 685
 Cys Arg Gln Ile Met Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser
 690 695 700
 Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val
 705 710 715 720
 His Gln Lys Ile Glu Val Lys Asp Thr Lys Glu Ala Leu Asp Lys Val
 725 730 735
 Glu Glu Glu Gln Asn Asn Ser Lys Lys Ala Gln Gln Glu Ala Ala
 740 745 750
 Asp Ala Gly Asn Arg Asn Gln Val Ser Gln Asn Tyr Pro Ile Val Gln
 755 760 765
 Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu
 770 775 780
 Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val
 785 790 795 800
 Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu
 805 810 815
 Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met
 820 825 830
 Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His
 835 840 845
 Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg
 850 855 860
 Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly
 865 870 875 880
 Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg
 885 890 895
 Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr
 900 905 910
 Ser Ile Leu Asp Ile Lys Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr
 915 920 925
 Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Thr Gln Glu
 930 935 940
 Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro
 945 950 955 960
 Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu
 965 970 975
 Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala
 980 985 990
 Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Gly Ser Ala Ala Ile
 995 1000 1005
 Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys

1010	1015	1020
Cys Phe Asn Cys Gly Lys Glu	Gly His Ile Ala Arg	Asn Cys Arg
1025	1030	1035
Ala Pro Arg Lys Lys Gly Cys	Trp Lys Cys Gly Lys	Glu Gly His
1040	1045	1050
Gln Met Lys Asp Cys Thr Glu	Arg Gln Ala Asn	
1055	1060	

<210> 42
<211> 1064
<212> PRT
<213> Artificial Sequence

<220>
<223> Hybrid protein comprised of Tat-Rev-Nef, CTL and truncated Gag protein(TRN-CTL-dgag)

<400> 42			
Met Ala Gly Arg Ser Gly Asp Ser Asp Glu Glu Leu Leu Lys Thr Val			
1	5	10	15
Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn Pro Pro Pro Ser Asn Glu			
20	25	30	
Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg Arg Arg Trp Arg Glu Arg			
35	40	45	
Gln Arg Gln Ile Arg Ser Ile Ser Glu Arg Ile Leu Ser Thr Phe Leu			
50	55	60	
Gly Arg Pro Ala Glu Pro Val Pro Leu Gln Leu Pro Pro Leu Glu Arg			
65	70	75	80
Leu Thr Leu Asp Cys Ser Glu Asp Cys Gly Asn Ser Gly Thr Gln Gly			
85	90	95	
Val Gly Ser Pro Gln Val Leu Val Glu Ser Pro Ala Val Leu Glu Pro			
100	105	110	
Gly Thr Lys Glu Thr Ser Val Gly Lys Trp Ser Lys Cys Ser Gly Trp			
115	120	125	
Pro Thr Val Arg Glu Arg Met Lys Gln Ala Glu Pro Glu Pro Ala Ala			
130	135	140	
Asp Gly Val Gly Ala Ala Ser Arg Asp Leu Glu Lys His Gly Ala Ile			
145	150	155	160
Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala Ala Cys Ala Trp Leu Glu			
165	170	175	
Ala Gln Glu Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro			
180	185	190	
Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu Asp Leu Ser His Phe Leu			
195	200	205	
Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile Tyr Ser Pro Lys Arg Gln			
210	215	220	
Glu Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp			
225	230	235	240
Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu Thr Phe			
245	250	255	
Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Asp Glu Glu Asn			
260	265	270	
Ser Ser Leu Leu His Pro Ala Ser Leu His Gly Thr Glu Asp Thr Glu			
275	280	285	
Arg Glu Val Leu Lys Trp Lys Phe Asp Ser His Leu Ala Phe His His			
290	295	300	
Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Lys Leu Glu			
305	310	315	320
Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser Gln Pro			
325	330	335	
Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Leu His Cys			
340	345	350	
Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly Arg Lys			

355	360	365
Lys Arg Arg Gln Arg Arg Arg Ala Pro Gln Asp Ser Gln Thr His Gln		
370	375	380
Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Gln Arg Gly Asp Pro Thr		
385	390	395
Gly Pro Lys Lys Ser Lys Lys Val Glu Arg Glu Thr Glu Ala Asp		
405	410	415
Pro Phe Asp Ala Ala Val Ile Thr Leu Trp Gln Arg Pro Leu Val Ala		
420	425	430
Leu Ile Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys		
435	440	445
Ile Gly Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp		
450	455	460
Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Lys Asp Phe Arg Lys		
465	470	475
Tyr Thr Ala Phe Thr Ile Pro Ser Ile Trp Lys Gly Ser Pro Ala Ile		
485	490	495
Phe Gln Ser Ser Met Thr Lys Lys Gln Asn Pro Asp Ile Val Ile Tyr		
500	505	510
Gln Tyr Met Asp Asp Leu Tyr Val Pro Ile Val Leu Pro Glu Lys Asp		
515	520	525
Ser Trp Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Ala Gly		
530	535	540
Ile Lys Val Lys Gln Leu Ile Leu Lys Glu Pro Val His Gly Val Tyr		
545	550	555
Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn		
565	570	575
Arg Ala Gly Asn Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp		
580	585	590
Lys Glu Ala Thr Thr Thr Leu Val Glu Arg Tyr Leu Arg Asp Gln Gln		
595	600	605
Leu Leu Gly Ile Trp Gly Cys Ala Cys Thr Pro Tyr Asp Ile Asn Gln		
610	615	620
Met Leu Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Arg Gln Gly Ser		
625	630	635
Leu Ala Ala Val Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly		
645	650	655
Gly Lys Lys Tyr Gln Leu Lys His Ile Val Trp Ala Ser Arg Glu		
660	665	670
Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly		
675	680	685
Cys Arg Gln Ile Met Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser		
690	695	700
Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val		
705	710	715
His Gln Lys Ile Glu Val Lys Asp Thr Lys Glu Ala Leu Asp Lys Val		
725	730	735
Glu Glu Glu Gln Asn Asn Ser Lys Lys Lys Ala Gln Glu Ala Ala		
740	745	750
Asp Ala Gly Asn Arg Asn Gln Val Ser Gln Asn Tyr Pro Ile Val Gln		
755	760	765
Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu		
770	775	780
Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val		
785	790	795
Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu		
805	810	815
Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met		
820	825	830
Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His		
835	840	845
Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg		
850	855	860

Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly
 865 870 875 880
 Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg
 885 890 895
 Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr
 900 905 910
 Ser Ile Leu Asp Ile Lys Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr
 915 920 925
 Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Thr Gln Glu
 930 935 940
 Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro
 945 950 955 960
 Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu
 965 970 975
 Glu Met Met Thr Ala Cys Gln Gly Val Gly Pro Gly His Lys Ala
 980 985 990
 Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Gly Ser Ala Ala Ile
 995 1000 1005
 Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys
 1010 1015 1020
 Cys Phe Asn Cys Gly Lys Glu Gly His Ile Ala Arg Asn Cys Arg
 1025 1030 1035
 Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His
 1040 1045 1050
 Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn
 1055 1060

<210> 43
 <211> 1064
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Hybrid protein comprised of Tat-Rev-Nef, truncated Gag protein and
 CTL (TRN-dgag-CTL)

<400> 43
 Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
 1 5 10 15
 Gln Pro Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Leu
 20 25 30
 His Cys Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly
 35 40 45
 Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro Gln Asp Ser Gln Thr
 50 55 60
 His Gln Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Gln Arg Gly Asp
 65 70 75 80
 Pro Thr Gly Pro Lys Lys Ser Lys Lys Val Glu Arg Glu Thr Glu
 85 90 95
 Ala Asp Pro Phe Asp Thr Ser Ala Gly Arg Ser Gly Asp Ser Asp Glu
 100 105 110
 Glu Leu Leu Lys Thr Val Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn
 115 120 125
 Pro Pro Pro Ser Asn Glu Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg
 130 135 140
 Arg Arg Trp Arg Glu Arg Gln Arg Gln Ile Arg Ser Ile Ser Glu Arg
 145 150 155 160
 Ile Leu Ser Thr Phe Leu Gly Arg Pro Ala Glu Pro Val Pro Leu Gln
 165 170 175
 Leu Pro Pro Leu Glu Arg Leu Thr Leu Asp Cys Ser Glu Asp Cys Gly
 180 185 190
 Asn Ser Gly Thr Gln Gly Val Gly Ser Pro Gln Val Leu Val Glu Ser
 195 200 205

Pro Ala Val Leu Glu Pro Gly Thr Lys Glu Lys Leu val Gly Lys Trp
 210 215 220
 Ser Lys Cys Ser Gly Trp Pro Thr Val Arg Glu Arg Met Lys Gln Ala
 225 230 235 240
 Glu Pro Glu Pro Ala Ala Asp Gly Val Gly Ala Ala Ser Arg Asp Leu
 245 250 255
 Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala
 260 265 270
 Ala Cys Ala Trp Leu Glu Ala Gln Glu Glu Glu Val Gly Phe Pro
 275 280 285
 Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu
 290 295 300
 Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile
 305 310 315 320
 Tyr Ser Pro Lys Arg Gln Glu Ile Leu Asp Leu Trp Val Tyr His Thr
 325 330 335
 Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val
 340 345 350
 Arg Tyr Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu
 355 360 365
 Pro Asp Glu Glu Asn Ser Ser Leu Leu His Pro Ala Ser Leu His
 370 375 380
 Gly Thr Glu Asp Thr Glu Arg Glu Val Leu Lys Trp Lys Phe Asp Ser
 385 390 395 400
 His Leu Ala Phe His His Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr
 405 410 415
 Lys Asp Cys Ala Ala Val Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg
 420 425 430
 Pro Gly Gly Lys Lys Tyr Gln Leu Lys His Ile Val Trp Ala Ser
 435 440 445
 Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser
 450 455 460
 Glu Gly Cys Arg Gln Ile Met Gly Gln Leu Gln Pro Ser Leu Gln Thr
 465 470 475 480
 Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr
 485 490 495
 Cys Val His Gln Lys Ile Glu Val Lys Asp Thr Lys Glu Ala Leu Asp
 500 505 510
 Lys Val Glu Glu Gln Asn Asn Ser Lys Lys Lys Ala Gln Gln Glu
 515 520 525
 Ala Ala Asp Ala Gly Asn Arg Asn Gln Val Ser Gln Asn Tyr Pro Ile
 530 535 540
 Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg
 545 550 555 560
 Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro
 565 570 575
 Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln
 580 585 590
 Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met
 595 600 605
 Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg
 610 615 620
 Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu
 625 630 635 640
 Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln
 645 650 655
 Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr
 660 665 670
 Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser
 675 680 685
 Pro Thr Ser Ile Leu Asp Ile Lys Gln Gly Pro Lys Glu Pro Phe Arg
 690 695 700
 Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Thr

705 710 715 720
 Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala
 725 730 735
 Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr
 740 745 750
 Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His
 755 760 765
 Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Gly Ser Ala
 770 775 780
 Ala Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val
 785 790 795 800
 Lys Cys Phe Asn Cys Gly Lys Glu Gly His Ile Ala Arg Asn Cys Arg
 805 810 815
 Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln
 820 825 830
 Met Lys Asp Cys Thr Glu Arg Gln Ala Asn Ala Ala Val Ile Thr Leu
 835 840 845
 Trp Gln Arg Pro Leu Val Ala Leu Ile Glu Ile Cys Thr Glu Met Glu
 850 855 860
 Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Ala Gly Leu Lys Lys Lys
 865 870 875 880
 Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro
 885 890 895
 Leu Asp Lys Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile
 900 905 910
 Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Lys Gln
 915 920 925
 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Pro
 930 935 940
 Ile Val Leu Pro Glu Lys Asp Ser Trp Leu Val Gly Lys Leu Asn Trp
 945 950 955 960
 Ala Ser Gln Ile Tyr Ala Gly Ile Lys Val Lys Gln Leu Ile Leu Lys
 965 970 975
 Glu Pro Val His Gly Val Tyr Glu Pro Ile Val Gly Ala Glu Thr Phe
 980 985 990
 Tyr Val Asp Gly Ala Ala Asn Arg Ala Gly Asn Leu Trp Val Thr Val
 995 1000 1005
 Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Thr Thr Leu Val
 1010 1015 1020
 Glu Arg Tyr Leu Arg Asp Gln Gln Leu Leu Gly Ile Trp Gly Cys
 1025 1030 1035
 Ala Cys Thr Pro Tyr Asp Ile Asn Gln Met Leu Arg Gly Pro Gly
 1040 1045 1050
 Arg Ala Phe Val Thr Ile Arg Gln Gly Ser Leu
 1055 1060

<210> 44
 <211> 1064
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Hybrid protein comprised of Rev-Nef-Tat, truncated Gag protein and
 CTL(RNT-dgag-CTL)

<400> 44
 Met Ala Gly Arg Ser Gly Asp Ser Asp Glu Glu Leu Leu Lys Thr Val
 1 5 10 15
 Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn Pro Pro Pro Ser Asn Glu
 20 25 30
 Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg Arg Arg Trp Arg Glu Arg
 35 40 45
 Gln Arg Gln Ile Arg Ser Ile Ser Glu Arg Ile Leu Ser Thr Phe Leu

50 55 60
 Gly Arg Pro Ala Glu Pro Val Pro Leu Gln Leu Pro Pro Leu Glu Arg
 65 70 75 80
 Leu Thr Leu Asp Cys Ser Glu Asp Cys Gly Asn Ser Gly Thr Gln Gly
 85 90 95
 Val Gly Ser Pro Gln Val Leu Val Glu Ser Pro Ala Val Leu Glu Pro
 100 105 110
 Gly Thr Lys Glu Thr Ser Val Gly Lys Trp Ser Lys Cys Ser Gly Trp
 115 120 125
 Pro Thr Val Arg Glu Arg Met Lys Gln Ala Glu Pro Glu Pro Ala Ala
 130 135 140
 Asp Gly Val Gly Ala Ala Ser Arg Asp Leu Glu Lys His Gly Ala Ile
 145 150 155 160
 Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala Ala Cys Ala Trp Leu Glu
 165 170 175
 Ala Gln Glu Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro
 180 185 190
 Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu Asp Leu Ser His Phe Leu
 195 200 205
 Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile Tyr Ser Pro Lys Arg Gln
 210 215 220
 Glu Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp
 225 230 235 240
 Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu Thr Phe
 245 250 255
 Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Asp Glu Glu Asn
 260 265 270
 Ser Ser Leu Leu His Pro Ala Ser Leu His Gly Thr Glu Asp Thr Glu
 275 280 285
 Arg Glu Val Leu Lys Trp Lys Phe Asp Ser His Leu Ala Phe His His
 290 295 300
 Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Lys Leu Glu
 305 310 315 320
 Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser Gln Pro
 325 330 335
 Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Leu His Cys
 340 345 350
 Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly Arg Lys
 355 360 365
 Lys Arg Arg Gln Arg Arg Ala Pro Gln Asp Ser Gln Thr His Gln
 370 375 380
 Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Gln Arg Gly Asp Pro Thr
 385 390 395 400
 Gly Pro Lys Lys Ser Lys Lys Val Glu Arg Glu Thr Glu Ala Asp
 405 410 415
 Pro Phe Asp Ala Ala Val Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg
 420 425 430
 Pro Gly Gly Lys Lys Tyr Gln Leu Lys His Ile Val Trp Ala Ser
 435 440 445
 Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser
 450 455 460
 Glu Gly Cys Arg Gln Ile Met Gly Gln Leu Gln Pro Ser Leu Gln Thr
 465 470 475 480
 Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr
 485 490 495
 Cys Val His Gln Lys Ile Glu Val Lys Asp Thr Lys Glu Ala Leu Asp
 500 505 510
 Lys Val Glu Glu Glu Gln Asn Asn Ser Lys Lys Lys Ala Gln Gln Glu
 515 520 525
 Ala Ala Asp Ala Gly Asn Arg Asn Gln Val Ser Gln Asn Tyr Pro Ile
 530 535 540
 Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg
 545 550 555 560

Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro
 565 570 575
 Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln
 580 585 590
 Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met
 595 600 605
 Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg
 610 615 620
 Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu
 625 630 635 640
 Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln
 645 650 655
 Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr
 660 665 670
 Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser
 675 680 685
 Pro Thr Ser Ile Leu Asp Ile Lys Gln Gly Pro Lys Glu Pro Phe Arg
 690 695 700
 Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Thr
 705 710 715 720
 Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala
 725 730 735
 Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr
 740 745 750
 Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Pro Gly His
 755 760 765
 Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Gly Ser Ala
 770 775 780
 Ala Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val
 785 790 795 800
 Lys Cys Phe Asn Cys Gly Lys Glu Gly His Ile Ala Arg Asn Cys Arg
 805 810 815
 Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln
 820 825 830
 Met Lys Asp Cys Thr Glu Arg Gln Ala Asn Ala Ala Val Ile Thr Leu
 835 840 845
 Trp Gln Arg Pro Leu Val Ala Leu Ile Glu Ile Cys Thr Glu Met Glu
 850 855 860
 Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Ala Gly Leu Lys Lys Lys
 865 870 875 880
 Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro
 885 890 895
 Leu Asp Lys Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile
 900 905 910
 Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Lys Gln
 915 920 925
 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Pro
 930 935 940
 Ile Val Leu Pro Glu Lys Asp Ser Trp Leu Val Gly Lys Leu Asn Trp
 945 950 955 960
 Ala Ser Gln Ile Tyr Ala Gly Ile Lys Val Lys Gln Leu Ile Leu Lys
 965 970 975
 Glu Pro Val His Gly Val Tyr Glu Pro Ile Val Gly Ala Glu Thr Phe
 980 985 990
 Tyr Val Asp Gly Ala Ala Asn Arg Ala Gly Asn Leu Trp Val Thr Val
 995 1000 1005
 Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala Thr Thr Leu Val
 1010 1015 1020
 Glu Arg Tyr Leu Arg Asp Gln Gln Leu Leu Gly Ile Trp Gly Cys
 1025 1030 1035
 Ala Cys Thr Pro Tyr Asp Ile Asn Gln Met Leu Arg Gly Pro Gly
 1040 1045 1050
 Arg Ala Phe Val Thr Ile Arg Gln Gly Ser Leu

1055 1060

<210> 45
<211> 1006
<212> PRT
<213> Artificial Sequence

<220>
<223> Hybrid protein cds comprised of Tat-Rev-Nef, truncated Gag protein
and CTL(TRN-optp17/24-CTL)

<400> 45
Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
1 5 10 15
Gln Pro Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Leu
20 25 30
His Cys Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly
35 40 45
Arg Lys Lys Arg Arg Gln Arg Arg Ala Pro Gln Asp Ser Gln Thr
50 55 60
His Gln Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Gln Arg Gly Asp
65 70 75 80
Pro Thr Gly Pro Lys Lys Ser Lys Lys Val Glu Arg Glu Thr Glu
85 90 95
Ala Asp Pro Phe Asp Thr Ser Ala Gly Arg Ser Gly Asp Ser Asp Glu.
100 105 110
Glu Leu Leu Lys Thr Val Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn
115 120 125
Pro Pro Pro Ser Asn Glu Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg
130 135 140
Arg Arg Trp Arg Glu Arg Gln Arg Gln Ile Arg Ser Ile Ser Glu Arg
145 150 155 160
Ile Leu Ser Thr Phe Leu Gly Arg Pro Ala Glu Pro Val Pro Leu Gln
165 170 175
Leu Pro Pro Leu Glu Arg Leu Thr Leu Asp Cys Ser Glu Asp Cys Gly
180 185 190
Asn Ser Gly Thr Gln Gly Val Gly Ser Pro Gln Val Leu Val Glu Ser
195 200 205
Pro Ala Val Leu Glu Pro Gly Thr Lys Glu Lys Leu Val Gly Lys Trp
210 215 220
Ser Lys Cys Ser Gly Trp Pro Thr Val Arg Glu Arg Met Lys Gln Ala
225 230 235 240
Glu Pro Glu Pro Ala Ala Asp Gly Val Gly Ala Ala Ser Arg Asp Leu
245 250 255
Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala
260 265 270
Ala Cys Ala Trp Leu Glu Ala Gln Glu Glu Glu Val Gly Phe Pro
275 280 285
Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu
290 295 300
Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile
305 310 315 320
Tyr Ser Pro Lys Arg Gln Glu Ile Leu Asp Leu Trp Val Tyr His Thr
325 330 335
Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val
340 345 350
Arg Tyr Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu
355 360 365
Pro Asp Glu Glu Glu Asn Ser Ser Leu Leu His Pro Ala Ser Leu His
370 375 380
Gly Thr Glu Asp Thr Glu Arg Glu Val Leu Lys Trp Lys Phe Asp Ser
385 390 395 400
His Leu Ala Phe His His Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr

Lys	Asp	Cys	Ala	Ala	Val	Gly	Ala	Arg	Ala	Ser	Val	Leu	Ser	Gly	Gly
405							410							415	
420							425							430	
Glu	Leu	Asp	Lys	Trp	Glu	Lys	Ile	Arg	Leu	Arg	Pro	Gly	Gly	Lys	Lys
435							440							445	
Lys	Tyr	Gln	Leu	Lys	His	Ile	Val	Trp	Ala	Ser	Arg	Glu	Leu	Glu	Arg
450							455							460	
Phe	Ala	Val	Asn	Pro	Gly	Leu	Leu	Glu	Thr	Ser	Glu	Gly	Cys	Arg	Gln
465							470							475	
Ile	Met	Gly	Gln	Leu	Gln	Pro	Ser	Leu	Gln	Thr	Gly	Ser	Glu	Glu	Leu
485							490							495	
Arg	Ser	Leu	Tyr	Asn	Thr	Val	Ala	Thr	Leu	Tyr	Cys	Val	His	Gln	Lys
500							505							510	
Ile	Glu	Val	Lys	Asp	Thr	Lys	Glu	Ala	Leu	Asp	Lys	Val	Glu	Glu	Glu
515							520							525	
Gln	Asn	Asn	Ser	Lys	Lys	Lys	Ala	Gln	Gln	Glu	Ala	Ala	Asp	Ala	Gly
530							535							540	
Asn	Arg	Asn	Gln	Val	Ser	Gln	Asn	Tyr	Pro	Ile	Val	Gln	Asn	Leu	Gln
545							550							555	
Gly	Gln	Met	Val	His	Gln	Ala	Ile	Ser	Pro	Arg	Thr	Leu	Asn	Ala	Trp
565							570							575	
Val	Lys	Val	Val	Glu	Glu	Lys	Ala	Phe	Ser	Pro	Glu	Val	Ile	Pro	Met
580							585							590	
Phe	Ser	Ala	Leu	Ser	Glu	Gly	Ala	Thr	Pro	Gln	Asp	Leu	Asn	Thr	Met
595							600							605	
Leu	Asn	Thr	Val	Gly	Gly	His	Gln	Ala	Ala	Met	Gln	Met	Leu	Lys	Glu
610							615							620	
Thr	Ile	Asn	Glu	Ala	Ala	Glu	Trp	Asp	Arg	Leu	His	Pro	Val	His	
625							630							635	
Ala	Gly	Pro	Ile	Ala	Pro	Gly	Gln	Met	Arg	Glu	Pro	Arg	Gly	Ser	Asp
645							650							655	
Ile	Ala	Gly	Thr	Thr	Ser	Thr	Leu	Gln	Glu	Ile	Gly	Trp	Met	Thr	
660							665							670	
Asn	Asn	Pro	Pro	Ile	Pro	Val	Gly	Glu	Ile	Tyr	Lys	Arg	Trp	Ile	Ile
675							680							685	
Leu	Gly	Leu	Asn	Lys	Ile	Val	Arg	Met	Tyr	Ser	Pro	Thr	Ser	Ile	Leu
690							695							700	
Asp	Ile	Lys	Gln	Gly	Pro	Lys	Glu	Pro	Phe	Arg	Asp	Tyr	Val	Asp	Arg
705							710							715	
Phe	Tyr	Lys	Thr	Leu	Arg	Ala	Glu	Gln	Ala	Thr	Gln	Glu	Val	Lys	Asn
725							730							735	
Trp	Met	Thr	Glu	Thr	Leu	Leu	Val	Gln	Asn	Ala	Asn	Pro	Asp	Cys	Lys
740							745							750	
Thr	Ile	Leu	Lys	Ala	Leu	Gly	Pro	Ala	Ala	Thr	Leu	Glu	Glu	Met	Met
755							760							765	
Thr	Ala	Cys	Gln	Gly	Val	Gly	Gly	Pro	Gly	His	Lys	Ala	Arg	Val	Leu
770							775							780	
Ala	Ala	Val	Ile	Thr	Leu	Trp	Gln	Arg	Pro	Leu	Val	Ala	Leu	Ile	Glu
785							790							795	
Ile	Cys	Thr	Glu	Met	Glu	Lys	Glu	Gly	Lys	Ile	Ser	Lys	Ile	Gly	Pro
805							810							815	
Ala	Gly	Leu	Lys	Lys	Lys	Ser	Val	Thr	Val	Leu	Asp	Val	Gly	Asp	
820							825							830	
Ala	Tyr	Phe	Ser	Val	Pro	Leu	Asp	Lys	Asp	Phe	Arg	Lys	Tyr	Thr	Ala
835							840							845	
Phe	Thr	Ile	Pro	Ser	Ile	Trp	Lys	Gly	Ser	Pro	Ala	Ile	Phe	Gln	Ser
850							855							860	
Ser	Met	Thr	Lys	Lys	Gln	Asn	Pro	Asp	Ile	Val	Ile	Tyr	Gln	Tyr	Met
865							870							875	
Asp	Asp	Leu	Tyr	Val	Pro	Ile	Val	Leu	Pro	Glu	Lys	Asp	Ser	Trp	Leu
885							890							895	
Val	Gly	Lys	Leu	Asn	Trp	Ala	Ser	Gln	Ile	Tyr	Ala	Gly	Ile	Lys	Val
900							905							910	

Lys Gln Leu Ile Leu Lys Glu Pro Val His Gly Val Tyr Glu Pro Ile
 915 920 925
 Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Ala Gly
 930 935 940
 Asn Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp Lys Glu Ala
 945 950 955 960
 Thr Thr Leu Val Glu Arg Tyr Leu Arg Asp Gln Gln Leu Leu Gly
 965 970 975
 Ile Trp Gly Cys Ala Cys Thr Pro Tyr Asp Ile Asn Gln Met Leu Arg
 980 985 990
 Gly Pro Gly Arg Ala Phe Val Thr Ile Arg Gln Gly Ser Leu
 995 1000 1005

<210> 46
<211> 1006
<212> PRT
<213> Artificial Sequence

<220>
<223> Hybrid protein cdscomprised of Tat-Rev-Nef, CTL and truncated Gag
protein (TRN-CTL-optp17/24)

<400> 46
Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
1 5 10 15
Gln Pro Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Leu
20 25 30
His Cys Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly
35 40 45
Arg Lys Lys Arg Arg Gln Arg Arg Ala Pro Gln Asp Ser Gln Thr
50 55 60
His Gln Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Gln Arg Gly Asp
65 70 75 80
Pro Thr Gly Pro Lys Ser Lys Lys Lys Val Glu Arg Glu Thr Glu
85 90 95
Ala Asp Pro Phe Asp Thr Ser Ala Gly Arg Ser Gly Asp Ser Asp Glu
100 105 110
Glu Leu Leu Lys Thr Val Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn
115 120 125
Pro Pro Pro Ser Asn Glu Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg
130 135 140
Arg Arg Trp Arg Glu Arg Gln Arg Gln Ile Arg Ser Ile Ser Glu Arg
145 150 155 160
Ile Leu Ser Thr Phe Leu Gly Arg Pro Ala Glu Pro Val Pro Leu Gln
165 170 175
Leu Pro Pro Leu Glu Arg Leu Thr Leu Asp Cys Ser Glu Asp Cys Gly
180 185 190
Asn Ser Gly Thr Gln Gly Val Gly Ser Pro Gln Val Leu Val Glu Ser
195 200 205
Pro Ala Val Leu Glu Pro Gly Thr Lys Glu Lys Leu Val Gly Lys Trp
210 215 220
Ser Lys Cys Ser Gly Trp Pro Thr Val Arg Glu Arg Met Lys Gln Ala
225 230 235 240
Glu Pro Glu Pro Ala Ala Asp Gly Val Gly Ala Ala Ser Arg Asp Leu
245 250 255
Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala
260 265 270
Ala Cys Ala Trp Leu Glu Ala Gln Glu Glu Glu Val Gly Phe Pro
275 280 285
Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu
290 295 300
Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile
305 310 315 320

Tyr Ser Pro Lys Arg Gln Glu Ile Leu Asp Leu Trp Val Tyr His Thr
 325 330 335
 Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val
 340 345 350
 Arg Tyr Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu
 355 360 365
 Pro Asp Glu Glu Asn Ser Ser Leu Leu His Pro Ala Ser Leu His
 370 375 380
 Gly Thr Glu Asp Thr Glu Arg Glu Val Leu Lys Trp Lys Phe Asp Ser
 385 390 395 400
 His Leu Ala Phe His His Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr
 405 410 415
 Lys Asp Cys Ala Ala Val Ile Thr Leu Trp Gln Arg Pro Leu Val Ala
 420 425 430
 Leu Ile Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys
 435 440 445
 Ile Gly Pro Ala Gly Leu Lys Lys Ser Val Thr Val Leu Asp
 450 455 460
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Lys Asp Phe Arg Lys
 465 470 475 480
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Trp Lys Gly Ser Pro Ala Ile
 485 490 495
 Phe Gln Ser Ser Met Thr Lys Lys Gln Asn Pro Asp Ile Val Ile Tyr
 500 505 510
 Gln Tyr Met Asp Asp Leu Tyr Val Pro Ile Val Leu Pro Glu Lys Asp
 515 520 525
 Ser Trp Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Ala Gly
 530 535 540
 Ile Lys Val Lys Gln Leu Ile Leu Lys Glu Pro Val His Gly Val Tyr
 545 550 555 560
 Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn
 565 570 575
 Arg Ala Gly Asn Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp
 580 585 590
 Lys Glu Ala Thr Thr Leu Val Glu Arg Tyr Leu Arg Asp Gln Gln
 595 600 605
 Leu Leu Gly Ile Trp Gly Cys Ala Cys Thr Pro Tyr Asp Ile Asn Gln
 610 615 620
 Met Leu Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Arg Gln Gly Ser
 625 630 635 640
 Leu Ala Ala Val Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu
 645 650 655
 Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr
 660 665 670
 Gln Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala
 675 680 685
 Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Met
 690 695 700
 Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser
 705 710 715 720
 Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Glu
 725 730 735
 Val Lys Asp Thr Lys Glu Ala Leu Asp Lys Val Glu Glu Glu Gln Asn
 740 745 750
 Asn Ser Lys Lys Ala Gln Gln Glu Ala Ala Asp Ala Gly Asn Arg
 755 760 765
 Asn Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln
 770 775 780
 Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys
 785 790 795 800
 Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser
 805 810 815
 Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn

820	825	830
Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile		
835	840	845
Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly		
850	855	860
Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala		
865	870	875
Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn		
885	890	895
Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly		
900	905	910
Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile		
915	920	925
Lys Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr		
930	935	940
Lys Thr Leu Arg Ala Glu Gln Ala Thr Gln Glu Val Lys Asn Trp Met		
945	950	955
Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile		
965	970	975
Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala		
980	985	990
Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu		
995	1000	1005

<210> 47

<211> 1006

<212> PRT

<213> Artificial Sequence

<220>

<223> Hybrid protein comprised of Rev-Nef-Tat, CTL and truncated Gag protein (RNT-CTL-optp17/24)

<400> 47	
Met Ala Gly Arg Ser Gly Asp Ser Asp Glu Glu Leu Leu Lys Thr Val	
1 5 10 15	
Arg Leu Ile Lys Phe Leu Tyr Gln Ser Asn Pro Pro Pro Ser Asn Glu	
20 25 30	
Gly Thr Arg Gln Ala Arg Arg Asn Arg Arg Arg Arg Trp Arg Glu Arg	
35 40 45	
Gln Arg Gln Ile Arg Ser Ile Ser Glu Arg Ile Leu Ser Thr Phe Leu	
50 55 60	
Gly Arg Pro Ala Glu Pro Val Pro Leu Gln Leu Pro Pro Leu Glu Arg	
65 70 75 80	
Leu Thr Leu Asp Cys Ser Glu Asp Cys Gly Asn Ser Gly Thr Gln Gly	
85 90 95	
Val Gly Ser Pro Gln Val Leu Val Glu Ser Pro Ala Val Leu Glu Pro	
100 105 110	
Gly Thr Lys Glu Thr Ser Val Gly Lys Trp Ser Lys Cys Ser Gly Trp	
115 120 125	
Pro Thr Val Arg Glu Arg Met Lys Gln Ala Glu Pro Glu Pro Ala Ala	
130 135 140	
Asp Gly Val Gly Ala Ala Ser Arg Asp Leu Glu Lys His Gly Ala Ile	
145 150 155 160	
Thr Ser Ser Asn Thr Ala Thr Asn Asn Ala Ala Cys Ala Trp Leu Glu	
165 170 175	
Ala Gln Glu Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro	
180 185 190	
Leu Arg Pro Met Thr Tyr Lys Gly Ala Leu Asp Leu Ser His Phe Leu	
195 200 205	
Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile Tyr Ser Pro Lys Arg Gln	
210 215 220	
Glu Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp	

225 230 235 240
 Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu Thr Phe
 245 250 255
 Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Asp Glu Glu Glu Asn
 260 265 270
 Ser Ser Leu Leu His Pro Ala Ser Leu His Gly Thr Glu Asp Thr Glu
 275 280 285
 Arg Glu Val Leu Lys Trp Lys Phe Asp Ser His Leu Ala Phe His His
 290 295 300
 Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Lys Leu Glu
 305 310 315 320
 Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser Gln Pro
 325 330 335
 Arg Thr Pro Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Leu His Cys
 340 345 350
 Gln Val Cys Phe Thr Arg Lys Gly Leu Gly Ile Ser Tyr Gly Arg Lys
 355 360 365
 Lys Arg Arg Gln Arg Arg Ala Pro Gln Asp Ser Gln Thr His Gln
 370 375 380
 Val Ser Leu Pro Lys Gln Pro Ser Ser Gln Gln Arg Gly Asp Pro Thr
 385 390 395 400
 Gly Pro Lys Lys Ser Lys Lys Val Glu Arg Glu Thr Glu Ala Asp
 405 410 415
 Pro Phe Asp Ala Ala Val Ile Thr Leu Trp Gln Arg Pro Leu Val Ala
 420 425 430
 Leu Ile Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys
 435 440 445
 Ile Gly Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp
 450 455 460
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Lys Asp Phe Arg Lys
 465 470 475 480
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Trp Lys Gly Ser Pro Ala Ile
 485 490 495
 Phe Gln Ser Ser Met Thr Lys Lys Gln Asn Pro Asp Ile Val Ile Tyr
 500 505 510
 Gln Tyr Met Asp Asp Leu Tyr Val Pro Ile Val Leu Pro Glu Lys Asp
 515 520 525
 Ser Trp Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Ala Gly
 530 535 540
 Ile Lys Val Lys Gln Leu Ile Leu Lys Glu Pro Val His Gly Val Tyr
 545 550 555 560
 Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn
 565 570 575
 Arg Ala Gly Asn Leu Trp Val Thr Val Tyr Tyr Gly Val Pro Val Trp
 580 585 590
 Lys Glu Ala Thr Thr Thr Leu Val Glu Arg Tyr Leu Arg Asp Gln Gln
 595 600 605
 Leu Leu Gly Ile Trp Gly Cys Ala Cys Thr Pro Tyr Asp Ile Asn Gln
 610 615 620
 Met Leu Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Arg Gln Gly Ser
 625 630 635 640
 Leu Ala Ala Val Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu
 645 650 655
 Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Tyr
 660 665 670
 Gln Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala
 675 680 685
 Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Met
 690 695 700
 Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser
 705 710 715 720
 Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Glu
 725 730 735

Val Lys Asp Thr Lys Glu Ala Leu Asp Lys Val Glu Glu Glu Gln Asn
 740 745 750
 Asn Ser Lys Lys Ala Gln Gln Glu Ala Ala Asp Ala Gly Asn Arg
 755 760 765
 Asn Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln
 770 775 780
 Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys
 785 790 795 800
 Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser
 805 810 815
 Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn
 820 825 830
 Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile
 835 840 845
 Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly
 850 855 860
 Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala
 865 870 875 880
 Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn
 885 890 895
 Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly
 900 905 910
 Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile
 915 920 925
 Lys Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr
 930 935 940
 Lys Thr Leu Arg Ala Glu Gln Ala Thr Gln Glu Val Lys Asn Trp Met
 945 950 955 960
 Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile
 965 970 975
 Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala
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 995 1000 1005

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<211> 1006

<212> PRT

<213> Artificial Sequence

<220>

<223> Hybrid protein comprised of Rev-Nef-Tat, truncated Gag protein and
CTL (RNT-optp17/24-CTL)

<400> 48

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 Gln Arg Gln Ile Arg Ser Ile Ser Glu Arg Ile Leu Ser Thr Phe Leu
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 Gly Arg Pro Ala Glu Pro Val Pro Leu Gln Leu Pro Pro Leu Glu Arg
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 Val Gly Ser Pro Gln Val Leu Val Glu Ser Pro Ala Val Leu Glu Pro
 100 105 110
 Gly Thr Lys Glu Thr Ser Val Gly Lys Trp Ser Lys Cys Ser Gly Trp
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 Pro Thr Val Arg Glu Arg Met Lys Gln Ala Glu Pro Glu Pro Ala Ala
 130 135 140

Asp Gly Val Gly Ala Ala Ser Arg Asp Leu Glu Lys His Gly Ala Ile
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 Thr Ser Ser Asn Thr Ala Thr Asn Ala Ala Cys Ala Trp Leu Glu
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 Ala Gln Glu Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro
 180 185 190
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 Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile Tyr Ser Pro Lys Arg Gln
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 Glu Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp
 225 230 235 240
 Trp Gln Asn Tyr Thr Pro Gly Pro Gly Val Arg Tyr Pro Leu Thr Phe
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 Ser Ser Leu Leu His Pro Ala Ser Leu His Gly Thr Glu Asp Thr Glu
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 Lys Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Lys Leu Glu
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<212> DNA
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Arg Val Tyr Ala Gly Val Ser Ser Thr Ser Ser Asp Phe Arg Asp Arg
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aaatttaggt

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 <213> Human herpesvirus 4

<400> 52
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 Lys Gly Asp Thr Ser Gly Pro Glu Gly Ser Gly Gly Ser Gly Pro Gln
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 35 40 45
 Arg Gly Gly Arg Pro Gly Ala Pro Gly Gly Ser Gly Ser Gly Pro
 50 55 60
 Arg His Arg Asp Gly Val Arg Arg Pro Gln Lys Arg Pro Ser Cys Ile
 65 70 75 80
 Gly Cys Lys Gly Thr His Gly Gly Thr Gly Ala Gly Ala Gly Ala Gly
 85 90 95
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 245 250 255
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 325 330 335
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 340 345 350
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 355 360 365
 Ala Arg Gly Arg Gly Arg Gly Arg Gly Glu Lys Arg Pro Arg Ser Pro
 370 375 380
 Ser Ser Gln Ser Ser Ser Ser Gly Ser Pro Pro Arg Arg Pro Pro Pro
 385 390 395 400
 Gly Arg Arg Pro Phe Phe His Pro Val Gly Glu Ala Asp Tyr Phe Glu
 405 410 415
 Tyr His Gln Glu Gly Gly Pro Asp Gly Glu Pro Asp Val Pro Pro Gly

Ala Ile Glu Gln Gly Pro Ala Asp Asp Pro Gly Glu Gly Pro Ser Thr	420	425	430
435	440	445	
Gly Pro Arg Gly Gln Gly Asp Gly Gly Arg Arg Lys Lys Gly Gly Trp			
450	455	460	
Phe Gly Lys His Arg Gly Gln Gly Gly Ser Asn Pro Lys Phe Glu Asn			
465	470	475	480
Ile Ala Glu Gly Leu Arg Ala Leu Leu Ala Arg Ser His Val Glu Arg			
485	490	495	
Thr Thr Asp Glu Gly Thr Trp Val Ala Gly Val Phe Val Tyr Gly Gly			
500	505	510	
Ser Lys Thr Ser Leu Tyr Asn Leu Arg Arg Gly Thr Ala Leu Ala Ile			
515	520	525	
Pro Gln Cys Arg Leu Thr Pro Leu Ser Arg Leu Pro Phe Gly Met Ala			
530	535	540	
Pro Gly Pro Gly Pro Gln Pro Gly Pro Leu Arg Glu Ser Ile Val Cys			
545	550	555	560
Tyr Phe Met Val Phe Leu Gln Thr His Ile Phe Ala Glu Val Leu Lys			
565	570	575	
Asp Ala Ile Lys Asp Leu Val Met Thr Lys Pro Ala Pro Thr Cys Asn			
580	585	590	
Ile Arg Val Thr Val Cys Ser Phe Asp Asp Gly Val Asp Leu Pro Pro			
595	600	605	
Trp Phe Pro Pro Met Val Glu Gly Ala Ala Ala Glu Gly Asp Asp Gly			
610	615	620	
Asp Asp Gly Asp Glu Gly Asp Gly Asp Glu Gly Glu Glu Gly Gln			
625	630	635	640
Glu			

INTERNATIONAL SEARCH REPORT

Int'l. application No.
PCT/FI 02/00379

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 15/86, C12N 15/63, C12N 15/85, A61K 48/00, A61P 31/00
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE, DK, FI, NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, PAJ, WPI-DATA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Virology, Volume 73, No. 5, 1999, Ivar Ilves et al: "Long-Term Episomal Maintenance of Bovine Papillomavirus Type 1 Plasmids Is Determined by Attachment to Host Chromosomes, Which Is Mediated by the Viral E2 Protein and Its Binding Sites", pages 4404-4412, page 4404 right column lines 12-35, page 4405 left column first paragraph, page 4409 left column third paragraph and page 4411 left column second paragraph lines 17-25 --	1-80
X	Eur. J. Biochem, Volume 267, 2000, Kathleen Van Craenenbroeck et al: "Episomal vectors for gene expression in mammalian cells", pages 5665-5678, page 5669, left column last paragraph - page 5672 right column second paragraph --	1-80

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 Sept 2002	04-09-2002
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer SARA NILSSON/BS Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 02/00379

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Vaccine, Volume 18, 2000, Auni Collings et al: "Humoral and cellular immune responses to HIV-1 Nef in mice DNA-immunised with non-replication of self-replicating expression vectors", pages 460-467, page 461 right column third paragraph and page 466 left column last paragraph --	1-80
X	Gene Therapy, Volume 4, 1997, S. Mücke et al: "Suitability of Epstein - Barr virus-based episomal vectors for expression of cytokine genes in human lymphoma cells", pages 82-92, page 85, figure 1(b) --	1-80
A	PNAS, Volume 93, Siu Chun Hung et al: "Maintenance of Epstein-Barr virus (EBV) oriP-based episomes requires EBV-encoded nuclear antigen-1 chromosome-binding domains, which can be replaced by high-mobility group-1 or histone H1, pages 1865-1870, page 1865, left column last paragraph, page 1869 right column paragraph 4 --	1-80
A	Virology, Volume 270, 2000, Nathalie Bastien et al: "Interaction of the Papillomavirus E2 with Mitotic Chromosomes", pages 124-134 --	1-80
A	WO 9807876 A2 (MEDICAL RESEARCH COUNCIL), 26 February 1998 (26.02.98) -----	1-80

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/FI02/00379**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: **53–58**
because they relate to subject matter not required to be searched by this Authority, namely:
see next sheet*
2. Claims Nos.: **1–80 partially**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see next sheet**
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI02/00379

*

Claims 53-58 relate to methods of treatment of the human or animal body by surgery or by therapy (Rule. 39.1.(iv)). Nevertheless, a search has been executed for these claims. The search has been based on the vector used and on the alleged effects of the vector.

**

Due to the wording "an expression vector **comprising...**", present claim 1 relate to an extremely large number of possible expression vectors. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the vectors claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts related to the vectors prepared in the examples. In these expression vectors, the nuclear-anchoring protein is the E2 protein of Bovine papilloma virus type 1 or Epstein-Barr virus nuclear antigen 1.

INTERNATIONAL SEARCH REPORT
Information on patent family members

06/07/02

International application No.
PCT/FI 02/00379

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9807876 A2	26/02/98	AT 199570 T	15/03/01
		AU 725474 B	12/10/00
		AU 4020997 A	06/03/98
		DE 69704206 D,T	30/08/01
		DK 918874 T	14/05/01
		EP 0918874 A,B	02/06/99
		SE 0918874 T3	
		ES 2157084 T	01/08/01
		GB 9617214 D	00/00/00
		JP 2000516472 T	12/12/00
		PT 918874 T	30/08/01

